At the beginning of life, the organisms obtained their energy (ATP) by anoxygenic photosynthesis, for which oxygen was toxic. Most of the metabolic pathways were developed during this anaerobic stage of life, in which oxygen came later. Cyanobacteria started producing oxygen from photosynthesis, which raised the atmospheric oxygen, and favored those organisms which have evolved into eukaryotic cells with mitochondria, able to use oxygen for a more efficient energy production (Naviaux RK, 2012).

Whenever a cell’s internal environment is perturbed by infections, disease, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus lowering oxygen consumption. This “oxidative shielding” acts as a defense mechanism for either decreasing cellular uptake of toxic pathogens or chemicals from the environment, or to kill the cell by apoptosis and thus avoid the spreading to neighboring cells (Naviaux RK, 2012). Therefore, ROS formation is a physiological response to stress.

2.1. OXIDATIVE STRESS

The term “oxidative stress” has been used to define a state in which ROS and RNS reach excessive levels, either by excess production or insufficient removal. Being highly reactive molecules, the pathological consequence of ROS and RNS excess is damage to proteins, lipids and DNA (Johansen JS, 2005).
Oxidative stress contributes to many pathological conditions, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, liver diseases, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and asthma. Aerobic organisms have integrated antioxidant systems which include enzymatic and non-enzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed (Birben E, 2012; Handa SS, 1989).

Oxidative process that is regularly going on in cell is essential for life and death of a cell. The following are the important key points taken into consideration:

- Molecular oxygen has ability to un-pair and leave free radicals which are unstable;
- This unstable radical is highly reactive and causes formation of reactive oxygen species;
- Beneficial biological functions such as apoptosis, necrosis, phagocytosis are mediated by reactive oxygen species;
- These reactive metabolites are selectively neutralized by body's defensive mechanism;
- Principal defensive agents are antioxidant enzymes and endogenous antioxidants;
- Balance is created between pro-oxidant and antioxidant in a cell, and any impairment in equilibrium causes deleterious effects on cell's life;
- Increased level of antioxidants may interfere the normal oxidative process;
- Decreased level of antioxidants generates reactive metabolites.

It is known that unpaired electron of molecular oxygen react to form highly reactive species, which are known as reactive oxygen species. Reactive oxygen species are generated from enzymatic and non-enzymatic sources (Orient A and Bedard K, 2007).

2.1.1. Non enzymatic sources

Fenton's & Haber's reactions: Fridovich (Fridovich I, 1984), Halliwell and Gutteridge (Halliwell B, 1989) explain the reduction of molecular oxygen to form superoxide
anions. These superoxide anions have ability to form more and highly reactive oxygen species. The dismutation of superoxides forms hydrogen peroxide.

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

Principal defensive agents are antioxidant H\textsubscript{2}O\textsubscript{2}, hydrogen peroxide, is more stable than O\textsubscript{2}- superoxide. It is permeable to plasma membrane and plays two important roles in the body. Either it is scavenged by catalase/GSH (reduced glutathione), or it helps in the formation of highly reactive oxygen species (Halliwell B, 1989).

Through the Fenton's reaction, these hydrogen peroxides react with metal iron or copper to form more highly reactive hydroxyl ions, OH-.

\[
\text{Fe}_2^+ + \text{H}_2\text{O}_2 \rightarrow \text{Fe}_3^+ + \text{OH}^- + \text{OH}^-
\]

Through Haber-Weiss reaction

\[
\begin{array}{c}
\text{O}_2^- + \text{H}_2\text{O}_2 \\
\text{Metal} \\
\text{Catalyst}
\end{array} \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}^-
\]

H\textsubscript{2}O\textsubscript{2} reacts with Cl\textsuperscript{-}, Br\textsuperscript{-}, I\textsuperscript{-} and is utilized by myloperoxidase to form more reactive hypochloric acid/hyperchlorite. This is important for protein aggregation and fermentation (Babior BM, 2000).

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

Formation of peroxynitrite is the primary reaction (Szabo C, 2003).

\[
\begin{array}{c}
\text{O}_2^- + \text{NO} \\
\end{array} \rightarrow \text{ONOO}^-
\]

Peroxynitrite is a very strong oxidant, which reacts with aromatic amino acid residues to form nitrotyrosine, which can lead to enzyme inactivation. To escape ROS, RNS
and lipid peroxidation dependence injury; biological structures have protective machinery in the form of endogenous antioxidants.

2.1.2. Enzymatic sources:

ROS are generated by oxygen metabolism, and have a single unpaired electron in their outer orbit that becomes highly reactive. It is produced in all aerobic organisms to perform cellular metabolisms. The enzymatic sources of ROS under subcellular levels are xanthine oxidases, cyclo-oxygenases (COX) and lipoxygenases (LOX), NO synthases (nitric oxide synthase) and mitochondrial oxidases (Lambeth JD, 2004).

2.1.2.1. Monoamine oxidase:

In mitochondrial membrane under the physiological condition, Electron transport chain produces ATP and O$_2^-$ . A heme containing enzyme monoamine oxidase present in outer mitochondrial membrane catalyzes oxidative deamination of amines, and thus produces H2O2 in matrix and cytosol (Cadenas E, 2000).

2.1.2.2. NADPH oxidase/Respiratory burst oxidase:

Phagocyte NADPH oxidase plays an important role in host defenses against invading microbes by generating superoxides. It is present in neutrophils and produces O$_2^{-}$. It is multicomplex enzyme located in plasma membrane of activated cell. It contains several components including cytochrome b558 (it is composed of gp91 phox, gp22 phox, phox phagocyte oxidase), p47, p67, p40 and rac 1 (monocytes)/rac 2 (neutrophils). The gp91 is bound to the cellular membrane. Upon stimulation, cytoplasmic subunits activate gp91 and cause respiratory bursts that activates superoxides, and releases them into the phagosomes (Prata C, 2006; El-Benna J, 2005).

In the same manner, endothelia, fibroblast, mesangial, osteoclast, chondrocytes and smooth muscles also generate superoxides, called NADPH-like oxidases which are activated by hormones and cytokines.
Besides being host defense, it also helps in signaling (Kerr S, 1999). ROS is produced by monovalent oxygen reduction.

\[ 2O_2 + NADPH \rightarrow NADPH \text{ Oxidase} \rightarrow 2O_2^- + NADP^+ + H^+ \]

### 2.1.2.3. Xanthine oxidoreductase:

This enzyme catalyzes hypoxanthine into xanthine, and then into uric acid. Xanthine Oxidoreductase (XOR) is present in the form of Xanthine Dehydrogenase (XD); these two forms of xanthine are transformed. XD is transformed into XO, irreversibly by proteolysis and reversibly by oxidation of sulfhydryls, and produce large amount of H2O2 and O2⁻. It is also found that XOR can transform nitrates into nitrites and NO. It also catalyzes the NO with O2⁻ and form highly reactive peroxynitrites (Cai H, 2000; Vorbach C and Gomez-Cabrera MC, 2003; Judge AR, 2004).

### 2.1.2.4. ROS generation by Arachidonic acid:

During the metabolism of arachidonic acid, ROS is generated intracellularly in which cyclooxygenase, lipoxygenase, cytochrome P450 oxidase enzymesystem are involved (Ivanov I, 2005).

### 2.1.2.5. Cytochrome P450 Oxidase:

This is heme-containing enzyme; it is present in mitochondria and participates in metabolism of cholesterol, steroids, hormones, catabolism of bile acids, arachidonic acid and eicosanoids, hydroxylation of vitamin D3, retinoid acid by catalyzing intramolecular transfer of oxygen. It transfers 2e⁻; one is bound to oxygen and the second is reduced to water. A part of oxygen is reduced to superoxides, inevitably.
2.1.2.6. Myeloperoxidase:

This is heme-containing enzyme, present in neutrophils and eosinophils and catalyzes the $\text{H}_2\text{O}_2$ with various substrates to form highly reactive hypochloric acids (Omura T, 1999; Klebanoff SJ, 2005).

2.1.3. NO and RNS production:

Nitric oxide $\bullet$NO is produced by the enzyme nitric oxide synthase (NOS), of which there are three isoforms: neural (nNOS or NOS-I) expressed in neurons, inducible (iNOS or NOS-II) expressed in smooth muscle of blood vessels, hepatocytes, macrophages and neuroendocrine tissue, and endothelial (eNOS or NOS-III) expressed constitutively in endothelial cells. iNOS and eNOS can be stimulated by the redox state in the cell, cytokines, hormones and nutrients (Schmidt HH, 1993; Hobbs AJ, 1996). NOS catalyze the oxidation of the terminal guanidine nitrogen of the L-arginine, in presence of oxygen and NADPH, to yield L-citruline and $\bullet$NO (Murad F, 1999).

Once produced and released, $\bullet$NO can diffuse freely through membranes or act on different cellular targets. $\bullet$NO participates as mediator of several physiological effects such as vasorelaxation, macrophage activation, gene expression and apoptosis. Usually, $\bullet$NO is considered as a vasculoprotective molecule. However, one of its multiple effects is also protein nitrosilation at the thiol groups and RNS generation such as peroxynitrite (ONOO$^-$), as $\bullet$NO easily reacts with $\bullet$O$_2^-$. Therefore, the amount of $\bullet$O$_2^-$ determines whether $\bullet$NO acts as a protective or harmful molecule (Johansen JS, 2005; Mc Donald LJ, 1995).

2.1.4. Function of ROS in Cells:

ROS performs beneficial functions in our body. Redox level should be maintained. It is the mediator of phagocytosis, apoptosis, detoxification reactions, executioner of precancerous cells and infections, etc. It is beneficially involved in signaling pathways to maintain cellular homeostasis in body. The ROS regulates many metabolic and
cellular processes including proliferation, migration, gene expression, immunity and wound healing (Salganik RI, 2001). Biochemical reactions are involved in the synthesis of prostaglandins, hydroxylation of proline and lysine, oxidation of xanthine and other oxidative processes (Spooner R, 2011).

2.1.5. Generation of ROS in Cells:

1. Mitochondria
2. Endoplasmic reticulum
3. Phagocytosis
4. Other sources

2.1.5.1. Production of ROS in mitochondria:
Electron transport chain produces superoxide anion in mitochondria by the reduction of molecular oxygen. ROS are generated by mitochondria, via the release of electrons from the electron transport chain and the reduction of oxygen molecules into superoxides (O$_2^-$). Superoxides, through the reaction catalyzed by superoxide dismutase (SOD), are transformed into the much less reactive hydrogen peroxide moiety (H$_2$O$_2$). However, when hydrogen peroxide interacts with ions of transition metals such as iron and copper, the most reactive ROS, hydroxyl radicals (OH-) are formed (Fenton's reaction) (Kowaltowski AJ, 2001).

\[
\begin{align*}
2O_2 + 2 H_2O & \rightarrow O_2 + H_2O_2 + 2OH^- \\
2H_2O_2 & \rightarrow 2H_2O + O_2^- \\
2H_2O_2 & \rightarrow \text{Fe}^{+2} \rightarrow \text{Fe}^{+3} \\
\cdot OH + OH^- & \rightarrow 2O_2
\end{align*}
\]
2.1.5.2. Production of ROS in endoplasmic reticulum:
Cytochrome P450 complexes are used to detoxify the toxic hydrophobic chemical compounds from the body; as a result, superoxide anions are formed. Enzyme cytochrome P450 reductase is used to detoxify into hydrophilic compounds. NADPH and NADH provide electrons for reduction of cytochrome b5 and cytochrome P450 (Bedard K, 2007).

2.1.5.3. Phagocytosis:
In phagocytosis, the bacteria when engulfed by the phagocytic cell results in the production of ROS. NADPH supplies electron and gives NADP+, hexose monophosphate shunt supplies energy to the NADP+ by NADPH oxidase through cytochrome b245 and form ROS. Bacteria and toxic cells are destroyed by respiratory burst produced by ROS (Ghosh MK, 1997).

2.1.5.4. Other sources:
Apoptosis: Apoptosis is a process of programmed cell death. It activates the Bcl-2; a group of protein that stimulates the Bax, which causes leakage of Cytochrome c. This Cyt c binds to Apaf-1 and form apoptosomes. This activates the caspase 9 and finally, causes protein denaturation and phagocytosis of the cell (Kam PC, 2000; Kerr JF, 1994).

Autooxidation of small molecules: Small molecules like dopamine, epinephrine, flavins and hydroquinones involve direct production of \( \text{O}_2^- \) (Freeman BA, 1982).

Peroxisomes generating hydrogen peroxide: Peroxisomes containing enzymes; glycolate oxidase, d – amino acid oxidase, urate oxidase, 1-\( \alpha \)-hydroxyacid oxidase, fatty acyl Co-A oxidase are involved in generating \( \text{H}_2\text{O}_2 \). The catalases involve in varieties of peroxidative reactions (Tolbert NE, 1981).
ROS Generation by Lysosomes: It carries electron transport chain which involves pumping of proton. This system promotes 3e⁻ reduction to oxygen and form highly reactive OH⁻ (Klebanoff SJ, 2005).

2.1.6. ROS Generation during the intercellular membrane

Catalytic reactions: Xanthine oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, flavoprotein dehydrogenase, tryptophan dioxygenase can generate ROS during catalytic cycling. Xanthine oxidase is formed from xanthine dehydrogenase, in hypoxic tissues and thus, generates O₂⁻ (Parks DA, 1988).

ROS production in non-phagocytic cells:

1. Phospholipid metabolism: membrane phospholipase A2 (PLA2) hydrolyzes the phospholipids to generate the Arachidonic acid. Arachidonic acid forms the 4 major classes of eicosanoids, which includes: prostaglandins, prostacyclins, thromboxanes and leukotrienes through the cyclooxygenase and lipoxygenase dependant synthesis. Their synthesizing steps involve the reactive intermediates.

2. Leukotriens derived oxygen species implicated redox status signaling by angiotensin II, IL-1 and epidermal growth factor. Angiotensin II induced O₂⁻ production in smooth muscle cell is also dependant on phospholipid pathway (Marumo T, 1998).

2.1.7. Receptors mediated ROS generation:

Ligand induced ROS is generated in nonphagocytic cells (Fanburg BL, 1997). NADPH oxidase is activated in vascular smooth muscle cells by Angiotensin II and 5-HT neurotransmitter. This in turn, generates hydrogen peroxides and superoxide anions. ROS activated by Ang-II stimulate the p38 Mitogen Activated Protein Kinase (MAPK) pathway and cause cell hypertrophy. ROS activated by 5-HT stimulate ERK (extra cellular signal regulated kinase), MAPK pathway and cause cell proliferation. So, ultimately 3 important sources of ROS generation are depicted:
a) Endogenous source

b) Exogenous source

c) Pathological source

a) **Endogenous Sources**: These are the products of important metabolic processes that are continuously going on in our body, e.g. detoxification reaction involves the P450 enzyme system and electron transport chain in mitochondria.

b) **Exogenous Sources**: Cigarette smoking, industrial waste products, ionization from radiations, ozone, asbestos fibers, viral & bacterial infections.

c) **Pathological Sources**: Radiation, immune cell activation, inflammation, ischemia, infections cancer, metabolism of environmental pollutants & certain drugs.

**Viral infections associated with reactive oxygen species:**

Many of the viral infections are associated with ROS generation, with total decreased level of antioxidants in intracellular and extracellular. Reactive oxygen species and reactive nitrogen intermediates possess antimicrobial and anti tumor activities, as well as they also participates in spreading of many pathological infections. It is known that viral infections interfere with the metabolic and physiologic mechanism of host cell (Peterhans E, 1997). Sendai and influenza viruses causes respiratory burst in phagocytic cells and thus, elevates the ROS/RNS concentration of the cell. Herpes viruses activate the host cell in early infections. Semiconfluent and proliferating cell are best for viral growth, while quiescent cell and confluent are not. Influenza virus exhibits ROS generation by using xanthine oxidase pathway and produce superoxides $O_2^-$, and host posses severe systemic symptoms like fever, myalgia, headache, anorexia. Virus bind with protease inhibitor present on cell membrane of lung surfactant and enter into the cell, where it is synthesizes HA0 (Haemagglutinin) as a precursor which is non-infectious.
This protein exhibits the proteolytic cleavage into HA1 and HA2; these are infectious strains. ROS increases in the lungs during infection, but inactivates the antiproteolytic activity of lung, therefore, causes inflammation and ultimately infection (Rott R, 1995; Kido H, 1993).

Similarly HIV (human immunodeficiency virus) increases the hydroperoxides and malonyldialdehyde (MDA) in human cells. HIV increases oxidative stress by stimulating transcription factor NFκB (Nuclear Factor κB), cytokines and TNF-α, which may result in release of H2O2 from T-Cells. The HIV virus and HIV gp120 attributes enhance production of NO in monocytes and causes neurotoxic effects (Chochola J and Westendorp MO, 1995).

Hepatitis virus directly affects the host genome and results in the production of RNS and ROS. It is characterized by increased cell proliferation, which ultimately becomes cancer (Ames BN, 1995).

Rabies, Lymphocytic choriomeningitis virus (Butz EA, 1994), bovine viral diarrhea viruses (Adler H, 1994), experimental allergic encephalomyelitis (Hooper DC, 1995), all exhibits increase production of NO. It causes immunosuppressive effect during the course of infections. Similarly, lentivirus and mycoplasma exhibits the NO mediated toxicity.

2.1.8. Pathways Involved in ROS Generation

ROS are generated through different cellular pathways including calcium dependant pathway, protein tyrosine kinase, protein tyrosine phosphatase, serine threonine kinase, phospholipase, mitogen activated protein kinase, NFκB, cytokines receptors, growth receptor, G–Protein coupled receptor, ion channel receptor and epidermal growth factor. These are briefly discussed.

Cytokines receptors:

IL-1, TNF-α, NK-κB are among the first receptors to generate ROS in non-phagocytic cells. TNF-α and oxidants may synergistically activate NK-κB by ROS independent mechanism. TNF-α generates mitochondrial ROS implicated in apoptotic cell death.
TNF-α activate IL-8, chemokines production, induction of cardiac myocytes hypertrophy by ROS dependant mechanism (Meier B, 1991).

Three different cells specific pathways of NK-kB have been evaluated, which is activated by IL-1β. ROS generated by 5-LOX in lymphoid cells, 5-LOX independent pathway in epithelial cells and NADPH oxidase dependent ROS production in monocytes (Bonizzi G, 1999). IFN-γ stimulates cyclooxygenase dependent peroxides in liver cell and exhibits antibacterial activity (Sundaresan M, 1995).

**Protein kinase receptors:**

For mitogenic signaling, a number of growth factors bind with protein kinase receptor for intra cellular ROS production. Fratti et al has demonstrated that platelet derived growth factor stimulates intracellular H$_2$O$_2$ (Fratti RA, 1996), required to induce tyrosine phosphorylation, DNA synthesis, chemotaxis, and MAPK activation (Krieger-Brauer HI, 1995). Epidermal growth factor (EGF) induces H2O2 formation by inhibition of protein tyrosine phosphatase activity. It is found that EGF stimulates intracellular O$_2^-$ by using LOX pathway. Heparin binding EGF stimulates ROS in vascular smooth muscle cells. EFG-induced peroxides production enhanced tumorigenicity, metastatic capacity. FGF-2 (Fibroblast growth factor-2) stimulates intracellular H2O2 by induction of c-Fos in chondrocytes, using flavoprotein oxidase (Thannickal VJ, 1995).

**Serine/Threonine kinase:**

These receptors are belonging to the TGF-β1 superfamily. Shibanuma et al found growth inhibitory effect on H$_2$O$_2$ from mouse osteoblastic cell(Shibanuma M, 1991). TGF-β1 inhibits the expression of SOD; catalase enzymes activity thus stimulates the oxidative stress. It also lowers the intracellular concentration of GSH glutathione in lung cell, primarily endothelial and epithelial cells. The prooxidants effect of TGF–β1 is growth inhibitory effect, apoptosis, TGF-β1 autoinduction, activation of latent TGF–β, cellular transformation, collagen synthesis, myofibroblast differenciation, etc (Shibanuma M, 1991).
G – Protein receptors:

Different ligands include angiotensin II, serotonin, 5-HT (5 Hydroxytryptamine), bradykinin, thrombin and endothelin generate ROS (Touyz RM, 1999). Ang II stimulates the ROS production in vascular smooth muscles cells (Lee SL, 1998). It is found that tyrosine phosphorylation of GTPase activating protein is induced by intracellular 5-HT, which generates superoxides by using mitogenic pathway through NADPH dependent oxidase activity (Holland JA, 1990). Bradykinin is vasodilator mediated by NO. ROS generated in endothelial cells is more likely through arachidonic acid metabolism, using cyclooxygenase pathway (Holland JA, 1998). Thrombin generates ROS in smooth muscle cell and endothelial cell using NADPH oxidase activity for mitogenesis (Sensi SL, 1999).

Ion channel receptors:

Neurotransmitters like 5-HT, acetylcholine, glutamate, glycine, γ – aminobutyric acid are mediated by signaling between the electrically excitable cells through ion channels (Reddy PH, 2011).

2.1.9. Pathophysiological role of ROS:

Oxidative stress has been linked to the pathogenesis of numerous diseases including asthma (mitochondrial dysfunction) (Vogiatzi G, 2009), atherosclerosis (oxidative modification of LDL) (Chen AF, 2012), endothelial cardiovascular disease, which is more prompt to inactivation of NO and ROS, thus predispose of these reactive molecules. The disease is characterized by altered anticoagulant and anti-inflammatory properties (Koevary SB, 2012), a cancer in which cells present mitochondria alterations at the level of mitochondrial DNA, oxidative phosphorylation and energy metabolism, all these activates the prooxidants and causes mitochondrial injury (Dell'Anna ML, 2010), inflammatory skin diseases in which Rho GTPases regulate the ROS production under the control of Rac protein in fibroblast, psoriasis and vitiligo (Chen H, 2012). Infertility to male due to impairment of spermatogenesis, as a result retention of cytoplasm in sperm midpiece causing increased activities of cytoplasmic enzymes like Glucose-6-Phosphate dehydrogenase,
which in turn produce more NADPH and finally increases the production of $O_2^-$ (ROS) (Singh RP, 2004).

Peroxynitrite reacts with body fluids and form nitrotyrosine in glial cell, which causes neurodegenerative diseases like Alzheimer's disease, in which Cu (I/II) involve the aggregation of amyloidogenic peptide with the production of Reactive Oxygen Species. Parkinson's disease is actually the neural degeneration due to the loss of pigmented neurons in substantia nigra, produces MPTP (Mitochondrial Permeability Transition Pore) which inhibits the complex I in Electron transport chain, thus results in decrease in the production of ATP.

Iron changes is seen in multiple sclerosis, spastic paraplegia in which iron causes secondary changes and accumulation of iron is related to gliosis, which produces ROS (Hegde KR, 2007). ROS is regulator of AIDS (Zhu DM, 2011), and cataract (Tkaczyk J, 2007).

ROS is also generated in type I pneumocytes on alveolar epithelium and causes destruction to cells. It is found that during inflammation, phagocytes are the main source of ROS generation (Inoguchi T, 2000). High glucose level in Diabetes and palmitic acid stimulate ROS through Protein kinase – C dependant NADPH oxidase in smooth muscle cells and endothelial cells (Hekimi S, 2003). A study showed the biology of ROS in mitochondria specifies the aging process in mutant stains of Caenorhabditis elegans (Olofsson P, 2003). Siegfried and Leonard (Hekimi S, 2003) found the gene SIR2 that exhibits the rate of aging. Arthritis severity is regulated by gene Ncf1 which reduced oxidative burst response and NADPH oxidase complex (Semchyshyn HM, 2011)

Oxygen free radicals need special attention as they are continuously being produced in organism and can cause damage to varying degrees until they are not efficiently scavenged.

Free radicals may define as any atom, group of atom or molecular in particular state with one or more unpaired electron occupying outer orbit. There are a number of intracellular sources for free radicals and ROS that have been identified(Fig. 1).
Figure 1: Cellular sources of free radicals produced by cells through action of various soluble and membrane bound enzymes (Kehrer, 1993).

ROS are highly reactive and unstable chemical species and enter into reaction in cell with biochemical species, particularly with key molecules in membranes and nucleic acid. There are five basic reaction characteristics of free radicals in biological system (Halliwell and Gutteridge, 1988; Machlin and Bendich, 1987; Sies, 1985) and are given below –

1. Hydrogen abstraction: \[ \text{A}^\cdot + \text{RH} \rightarrow \text{AH} + \text{R}^\cdot \]

2. Electron transfer: \[ \text{X}^\cdot + \text{Y} \rightarrow \text{X} + \text{Y}^\cdot \]

3. Addition: \[ \text{X} + \text{RCH} = \text{CHR} \]

4. Termination: \[ \text{A} + \text{A} \rightarrow \text{A}_2 \]

5. Disproportionation: \[ \text{CH}_2\text{CH}_2 + \text{CH}_3\text{CH}_2 \rightarrow \text{CH}_3\text{CH}_3 + \text{CH}_2=\text{CH}_2 \]
Free radicals are now recognized as an important mediator of tissue damage (Sinclair, 1991; Reilly and Bulkley, 1990). These free radicals cause fragmentation, cross linking and aggregation of protein. In hyperglycemia, there is a tendency for non enzymatic linkage of carbohydrates to protein by process called glycation. This leads to cytotoxicity, mutation and oncogenic potential (Sharma, 1994). Exposure of tissues to free radicals may cause interaction with proteins, lipids, DNA, carbohydrates and cause changes that affect the cell function (Chance, 1979)(Fig. 2).

![Mechanism of free radicals in cell damage/injury](image)

**Figure 2: Mechanism of free radicals in cell damage/injury (Bulkley, 1990).**

**Carbohydrates oxidation by ROS:**

Free carbon and hydrogen of deoxy sugars are attributed to the oxidation of carbohydrates, e.g. mannitol and glucose. The free radicals binds with these carbohydrates and forms carbon centered radicals. These carbon centered radicals interacts with other carbohydrates, and thus series of autocatalytic chain reaction
commence resulting in the destruction of the cells. Ketoamines and ketoaldehydes are the most common oxidative products of carbohydrates (Garrison WM, 1987).

**Protein oxidation by ROS:**

Reactive oxygen species interacts on protein molecules at the specific amino acid side chain and form the modification in protein structure results fragmentation of the peptide chain, alteration in electrical charges; peroxynitrite nitrate protein is accumulated and thus, increases the proteolysis. Garrison (Garrison WM, 1987) has found that active oxygen has potential to react with amino acid side groups and cleaving the polypeptide chain, thus resulting in the formation of reactive carbonyl groups (Stadtman ER, 1991; Kaur S, 2011). Stadtman and Oliver (Stadtman ER, 1991) proposed the protein oxidation mechanism in which lysresidue is converted to α amino-adipic semialdehyde. He found that ferrous ions formed by reduction through superoxide anion from ferric ion bind to cationic side of amino acid on protein molecule, in which one amino acid is lys. This bound metal react with hydrogen peroxide and form hydroxylradical, which help in production of carbonyl radical. This radical inturn cleaves the polypeptide chain. Oxidative markers are proteincarbonyl groups (Blokhina O, 2003).

**Lipid peroxidation by ROS:**

Oxidative stress causes damage to cellular macromolecules such as nucleic acids, proteins, and lipids. Among these targets, the peroxidation of lipids is particularly more damaging because the formation of lipidperoxidation products leads to a facile propagation of free radical reactions. Abstraction of a hydrogen atom from the Poly Unsaturated Fatty Acid (PUFA) moiety of membrane phospholipids initiates the process of lipid peroxidation. Alkyl radicals are formed which areultimately rearranged to form conjugated diens, and stimulates theautocatalytic lipid peroxidation cascades. ROS directly attacks onphospholipid hydroperoxides and fatty acid hydroperoxides.
The fatty acid carbon chain spontaneously was cleaved during lipid peroxidation process and yield highly toxic pentane, ethane, α, β unsaturated (fattynonenal) are the potent aldehydic lipid peroxidation products of ω3 and ω6 PUFA. The accepted markers for oxidative stress are aldehydic secondary products MDA and 4-HNE (Mattill HA, 1947).

**Oxidation of nucleic acid/DNA by ROS:**

ROS break the DNA strands, forms DNA adduct which is characterized by deletion, mutation and causes genetic effects. Sugars and base moieties are degraded by ROS and causes oxidation of bases and cross linking to protein. 8-hydroxyguanine, hydroxyl methyl urea, urea, thymine, and saturated products which are the oxidation of bases; polyadenosine diphosphate ribose synthesis in nuclei resulting in extensive depletion of cellular NADH pools is the DNA oxidation products. DNA-MDA adducts is the most characteristic feature of nucleic acid oxidation. The measuring oxidative marker is 4-hydroxyl, 2-deoxyguonosine, which is the oxidative marker of DNA oxidation (Moreau, 1922).

Thus, oxidative stress is ultimately defined as "the imbalances in the equilibrium between pro-oxidants/antioxidants status in cellular systems, which results in damaging the cells." Cells have an intact oxidation process to detoxify the cellular environment from oxidants, and thus create the equilibrium in oxidants and antioxidants from aerobic metabolism. The formation of pro-oxidants is readily balanced by antioxidants by a similar rate. The failure in the neutralization events of oxidative status result in oxidative stress which leads to the cell death by lipid peroxidation, carbohydrates oxidation, protein oxidation and nucleic acid oxidation.
The pancreas is both an endocrine gland that produces the peptide hormones insulin, glucagons and somatostatin, and an exocrine gland that produces digestive enzymes. The peptide hormones are secreted from cells located in the islets of Langerhans (β- or B-cells produce insulin, α2- or A-cells produce glucagon, and α1- or D-cells produce somatostatin). These hormones play an important role in regulating the metabolic activities of the body and in doing so; help maintain the homeostasis of blood glucose. Hyperinsulinemia can cause severe hypoglycemia. More commonly, a relative or absolute lack of insulin can cause serious hyperglycemia (Mycek M, 1997)

2.2. DIABETES MELLITUS

Diabetes mellitus is a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular diseases (Goodman & Gillman, 2003). Diabetes is a chronic incurable condition. Diabetes needs proper diagnosis, care and diet but, with less chance of complete eradication (Kakuda T et al., 1996).

Terminology

The term 'diabetes' was coined by Aretaeus of Cappadocia. Greeks named the symptom of frequent urination as diabetes. In Greek the word diabetes means “siphon” representing the large quantities of fluid discharge in the victims of this disease. The Romans observed that the urine of the people affected with this disease is sweet and they added the Latin word “mellitus” to diabetes which means “honey”. In 1675 Thomas Willis rediscovered the sweetness of urine and blood of the patients affected with diabetes (Ayashi T et al., 2002; Anthony M, 2002).

History

Diabetes is an ancient disease with symptoms like excess drinking of water and frequent urination which are noted on a scrap of Egyptian papyrus more than 3,500
years ago. Since that time many physicians tried to make the study against the sweet
taste of the diabetes urine. Therefore the technical term like “sweet flow or siphon” is
kept for the disease and because of this for more than 2000 years diabetes was thought
to be the disease of kidney and bladder as the frequent urination is the main symptom
of the disease. The Chemistry knowledge in 17th century made the scientists like
Matthew Dobson to extract sugar molecule from the urine and blood of the diabetic
patients.

Then the French scientist Chevruel shown that the sugar more extracted from the
urine and blood of the diabetic patients is similar to glucose. In 1796 John Roll
became the first person to show that the belief which was made earlier that kidney, the
main organ that is involved in the diabetes was denied by saying that the urine
glucose level depends upon the type and amount of food material taken and also on
the basis of glycosuria secreted by the diabetic patient (Anthony M, 2002).

Oskar Minkowski and Joseph von Mering first discovered the critical role of pancreas
in diabetes by an experiment where they removed the pancreas of dog and observed
the diabetic symptoms followed by the death of the animal (Anthony M, 2002). Dr.
Banting with the assistance of Best, colleagues (particularly Collip) continued his
research on de-pancreatized dogs, but went a step further and demonstrated that they
could reverse induced diabetes in dogs by giving them an extract from the pancreatic
islets of Langerhans of healthy dogs. For this, Banting et al received the Nobel Prize
in Physiology or Medicine in 1923.

After the discovery of insulin by Banting and best many experiments have been
carried to confirm that the diabetes is mainly due to the lack of insulin. In 1930 the
British clinician Harry Himsworth thought about the effects of diet on sensitivity of
insulin with a series of experiments and finally concluded that the use of sugar by the
body not only depends upon the amount of insulin present in the body but also the
sensitivity of the body to the effects of insulin. This shown that the diabetes occurs
may be with one of the reasons either due to lack of insulin or due to insensitivity of
the body to the insulin. Himsworth’s experiments showed that there were two types of
diabetes: type-1 and type-2. People with type-1 diabetes were sensitive to insulin and
occurred at young age with type – 2 were insensitive to insulin and tended to gradually develop a milder form of disease at middle age or older (Patlak M, 2002).

Other landmark discoveries include (Anthony M, 2002):

1. Sulfonylurea was discovered by the chemist Marcel Janbon and co-worker.
2. The radioimmunoassay for insulin, discovered by Rosalyn Yalow and Solomon Berson (gaining Yalow the 1977 Nobel Prize in Physiology or Medicine).

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration – hyperglycaemia (fasting plasma glucose > 7.0 mmol/l or plasma glucose > 11.1 mmol/l 2 hours after a meal) – caused by insulin deficiency, often combined with insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydipsia) (Rang HP, 2003).

Diabetes mellitus also means idiopathic diabetes mellitus. Secondary diabetes mellitus is occurrence of hyperglycaemia associated with some identifiable causes such as due to choronic pancreatitis, post-pancratectomy, hormone-producing tumours, certain drugs, haemochromatosis and genetic endocrinologic disorder (Mohan H, 2000)

The number of cases of diabetes worldwide in 2000 among adults 20 years of age is estimated to be 171 million. This figure is 11% higher than the previous estimate of 154 million. The IDF (International Diabetes federation) has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively (Wild S, 2004).

According to WHO:

- More than 220 million people worldwide have diabetes.
- In 2005, an estimated 1.1 million people died from diabetes.
- Almost 80% of diabetes deaths occur in low- and middle-income countries.
Almost half of diabetes deaths occur in people under the age of 70 years and 55% of diabetes deaths are in women.

WHO projects that diabetes death will double between 2005 and 2030.

This increase in diabetes, results from multiple factors involved in lifestyle changes related to modern life such as the decrease in physical activities and the predominance of hyper-caloric diets and the resulting obesity. Also playing a major part is the aging process of the population in developing countries. Given these various factors, an increase in diabetes mellitus caseload will be more evident in developing countries (Alberto B, 2001). At the dawn of the 21st century, diabetes mellitus, especially type 2 diseases, has assumed epidemic proportions and has become the most prevalent chronic disease, and certainly the most costly public health problem, facing the developed countries. Also prevalence of diabetes is assumed to be similar in urban and rural areas in developed countries. It is also growing at an alarming rate in the developing nations (Zimmet PM, 1997).

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “diabetes capital of the world”. According to the Diabetes Now here is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that (Fig. 3) 32 million people had diabetes in the year 2000. International Diabetes Federation, (2006) published the number of people with diabetes in India currently is around 40.9 million and is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken (Mohan V, 2007).

<table>
<thead>
<tr>
<th>Year</th>
<th>Estimated number of Diabetic subjects in India</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>32 million</td>
</tr>
<tr>
<td>2006</td>
<td>40.9 million</td>
</tr>
<tr>
<td>2025</td>
<td>69.9 million</td>
</tr>
<tr>
<td>2030</td>
<td>80 million</td>
</tr>
</tbody>
</table>

Figure 3: Estimated number of Diabetic subjects in India (Mohan V, 2007).
The term diabetes mellitus was recognized as ‘Madhumeha’ in primeval times. Our ancient Hindu Physicians had mastered the science of managing this disorder with effective balance of “Aushada” some herbs or plant food sources) as medicine, “Ahar” (Pathyam) in modern terms therapeutic diets and ‘Vihar’ (exercise).

Those indigenous foods alone may not be as effective as insulin in lowering the blood sugar but the combination therapy seems to equate with the modern methods of drug, diet and exercise. Mankind has a long history in the use of herbal medicines. Rigveda and Ayurveda (4500 – 1600 BC) reveal that ancient Indians had a rich knowledge of the use of medicinal plants. India unquestionably occupies the topmost position in the use of herbal drugs since ancient times utilizing nearly 600 plant species in different formulations. Great majorities of people in India have been depending on crude drugs

**Figure 4:** Recent population based studies showing the prevalence of type 2 diabetes in different parts of India (Mohan V, 2007).
for the treatment of various diseases as evidenced from well-documented indigenous system of medicines, Ayurveda and Unani. The Materia Medica of these systems contains a rich heritage of indigenous herbal drugs (Subbalakshimi G, 2001).

Treatment of type-I diabetes comprises insulin therapy & type-II with oral hypoglycemic in the initial phases & then a combination of insulin and oral hypoglycemic in the later phase.

Main drawback is that all of these allopathic medicinal agents keep blood sugar level in normal range for only few hours and they have many side effects including hematological effects, coma and disturbances of the liver and kidney. In addition, they are not suitable for use during pregnancy (Gilman AG, 1985). Also synthetic drugs possess antidiabetic activity but have lack of antioxidant activity and antioxidant plays a key role in treatment of both types of diabetes mellitus. Therefore the search for more effective and safer antihyperglycemic and antioxidant agents have become an area of current research.

In search of alternative treatment of diabetes, today world is moving toward the herbal options. Today plant based drugs have been in use against various diseases since long time. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plants have been published but a large number of them remain unexplored as yet. Many antidiabetics herbs are used in many traditional system of medicine. Many traditional plant medicines are used worldwide for range of diabetes preparations. In Ayurveda, there are descriptions of various part of plant which have antidiabetic properties.

These indigenous plants were used as traditional phytotherapies for the control and treatment of diabetes mellitus. The rural inhabitants of the area classified these traditional phytotherapies on the basis of uses of various parts of plants and method of their uses. About 29 traditional phytotherapies were investigated from the rural inhabitants of the area. These traditional phytotherapies were classified as antidiabetic extracts, leaves, powders, seeds, vegetables, fruits and herbal mixtures.

Despite considerable progress in the management of diabetes mellitus by conventional synthetic drugs, the search for natural anti-diabetic plant products for controlling
diabetes is going on. Hypoglycemic activity has been reported in many plants during the last twenty years.

The use of local plants for treatment of diabetes mellitus is quite common in Asia and Middle East countries. More than 400 local plants are used for the treatment for diabetes mellitus. Approximately 343 plants of the world have been tested for the blood glucose lowering effect in the laboratory experiments (Ajgaonkar SS, 1979). Of these plants 158 are claimed to be used in the Ayurveda (Rahman AU, 1989).

2.2.1. The pancreas:

Pancreas is a flattened elongated organ lying against the posterior wall of the upper abdomen. The vast bulk of the gland consists of acinar cells that synthesize and secrete digestive enzymes that enter the duodenum via the pancreatic duct system, performing the exocrine function. Scattered more or less randomly through the pancreas and accounting for only 1-2% of its weight are several microscopic nests of cells, the “Islets of langerhans”, which collectively constitute the endocrine pancreas, secreting several critically important hormones directly into the blood stream (West J.B, 1990).

Pancreas of human beings has 1-2 million islets of langerhans, each having a diameter of 0.3mm and organized in around small capillaries in to which its cells secrete hormones (Guyton AC, 1998).

Each pancreatic islet includes four types of hormone secreting cells;

1. **Alpha (α) or A cells** constitute about 20% of pancreatic islet cells and secrete glucagon
2. **Beta (β) or B cells** constitute about 70% of pancreatic islet cells and secrete insulin
3. **Delta (δ) cells or D cells** constitutes about 5% of pancreatic islet cells and secrete somatostatin and
4. **F cells** constitute the remainder of pancreatic islet cells and secrete pancreatic polypeptide (Tortora GT, 1996) which regulates release of pancreatic digestive enzyme.

Two minor cell types, called D1 cells and enterochromaffin cells are also seen in islets. D1 cells elaborate vasoactive intestinal polypeptide, a hormone that induces glycogenolysis and hyperglycemia; it also stimulates gastrointestinal fluid secretion and causes secretory diarrhea. Enterochromaffin cells synthesize serotonin and are the source of pancreatic tumours (Cotran RS, 2000).

![Gross and Microscopic Anatomy of Pancreas](image)

**Figure 5: Gross and Microscopic Anatomy of Pancreas (Bardeesy N, 2002).**

- a] Gross anatomy of pancreas
- b] The exocrine pancreas
- c] A single acinus
- d] A pancreatic islets embedded in exocrine tissue

2.2.2. **INSULIN:**

Insulin is discovered in 1921 by Banting and Best who demonstrated the hypoglycemic action of an extract of pancreas prepared after degeneration of the exocrine part due to ligation of pancreatic duct. It was first obtained in pure...
crystalline form in 1926 and the chemical structure was fully worked out in 1956 by
Sanger (Tripathi KD, 2003).

Insulin is a small protein consisting of two polypeptide chains that are connected by
disulfide bonds. It is synthesized as a precursor protein (pro-insulin) that undergoes
proteolytic cleavage to form insulin and peptide C, both of which are secreted by the
β-cells of the pancreas. Not only blood glucose, other hormones and autonomic
mediators also regulate the insulin secretion. Secretion is most commonly triggered by
high blood glucose which is taken up and phosphorylated in the β-cells of the
pancreas. Adenosine triphosphate (ATP) levels rise and block K+ Channels, leading
to membrane depolarization and an influx of Ca++, which causes pulsatile insulin
exocytosis. Glucose given by injection has a lower effect on insulin secretion than
glucose taken orally, because orally taken glucose stimulates production of digestive
hormones by the gut, which in turn stimulates insulin secretion by the pancreas.
Because insulin is a protein, it is degraded in the gastrointestinal tract if taken orally.
It is therefore generally administered by subcutaneous injection. Insulin is inactivated
by the reducing enzyme insulinase, found mainly in the liver and kidney (Myczek M,
1997).

2.2.3. Classification of Diabetes mellitus:

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)

   A. Immune mediated       B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative
    insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types

A. Genetic defects of β-cell function

   1. Chromosome 12, HNF-1α (MODY3)

   2. Chromosome 7, glucokinase (MODY2)
3. Chromosome 20, HNF-4 α (MODY1)

4. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY4)

5. Chromosome 17, HNF-1 β (MODY5)

6. Chromosome 2, NeuroD1 (MODY6)

7. Mitochondrial DNA

8. Others

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipoatrophic diabetes

5. Others

C. Diseases of the exocrine pancreas

1. Pancreatitis

2. Trauma/pancreatectomy

3. Neoplasia

4. Cystic fibrosis

5. Hemochromatosis

6. Fibrocalculous pancreatopathy

7. Others

D. Endocrinopathies

1. Acromegaly
2. Cushing’s syndrome
3. Glucagonoma
4. Pheochromocytoma
5. Hyperthyroidism
6. Somatostatinoma
7. Aldosteronoma
8. Others

E. Drug- or chemical-induced

1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. β-adrenergic agonists
8. Thiazides
9. Dilantin
10. α-Interferon
11. Streptozotocin
12. Others

F. Infections

1. Congenital rubella
2. Cytomegalovirus

3. Others

G. Uncommon forms of immune-mediated diabetes

1. ‘Stiff-man’ syndrome

2. Anti-insulin receptor antibodies

3. Others

H. Other genetic syndromes sometimes associated with diabetes

1. Down’s syndrome

2. Klinefelter’s syndrome

3. Turner’s syndrome

4. Wolfram’s syndrome

5. Friedreich’s ataxia

6. Huntington’s chorea

7. Laurence-Moon-Biedl syndrome

8. Myotonic dystrophy

9. Porphyria

10. Prader-Willi syndrome

11. Others

IV. Gestational diabetes mellitus
I. Type 1 Diabetes:

A. Immune-mediated diabetes:

This form of diabetes, previously encompassed by the terms insulin-dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and B genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults).

Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years. Many such individuals with this form of type 1 diabetes eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C peptide. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Grave’s disease, Hashimoto’s thyroiditis, Addison’s disease, vitiligo, and pernicious anemia.
B. Idiopathic diabetes:

Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian origin. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes.

This form of diabetes is strongly inherited, lack immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go.

Pathogenesis of Type-I diabetes mellitus (James MC, 2000): Three interlocking mechanisms responsible for the islet cell destruction are:

1. Genetic Susceptibility
2. Auto-Immunity
3. Environmental
II. Type 2 Diabetes:

This form of diabetes, previously referred to as non-insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, is a term used for individuals who have insulin resistance and usually have relative insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive.

There are probably many different causes of this form of diabetes, and it is likely that the proportion of patients in this category will decrease in the future as identification...
of specific pathogenic processes and genetic defects permits better differentiation among them and a more definitive sub classification.

Although the specific etiologies of this form of diabetes are not known, autoimmune destruction of \( \beta \)-cells does not occur, and patients do not have any of the other causes of diabetes listed above or below. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance.

Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection.

This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their \( \beta \)-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for the insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal.

**Pathogenesis of Type-II diabetes mellitus:**

The two metabolic defects that characterize Type-II diabetes mellitus are:

1. A derangement in \( \beta \)-cell secretion of insulin
2. A decrease response of peripheral tissue to respond to insulin (Insulin resistance)
   (Rang HP, 1999)
The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type I diabetes. However, the genetics of this form of diabetes are complex and not clearly defined.

III. Other specific types of diabetes:

A. Genetic defects of the β-cell function:

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity onset diabetes of the young and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant
Abnormalities at three genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as HNF-1\(\alpha\).

A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the \(\beta\)-cell. Thus, glucokinase serves as the “glucose sensor” for the \(\beta\)-cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. A third form is associated with a mutation in the HNF-4\(\alpha\) gene on chromosome 20q. HNF-4\(\alpha\) is a transcription factor involved in the regulation of the expression of HNF-1\(\beta\). The specific genetic defects in a substantial number of other individuals who have a similar clinical presentation are currently unknown.

Point mutations in mitochondrial DNA have been found to be associated with diabetes mellitus and deafness. The most common mutation occurs at position 3243 in the tRNA leucine gene, leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome; however, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion. Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families, and such traits are inherited in an autosomal dominant pattern. The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism.

B. Genetic defects in insulin action:

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the
Rabson-Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance.

The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia. Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin resistant lipoatrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the post receptor signal transduction pathways.

C. Diseases of the exocrine pancreas:

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion.

Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications on X-ray. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy.

D. Endocrinopathies:

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing’s syndrome, glucagonoma, pheochromocytoma) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is removed.

Somatostatinoma and aldosteronoma-induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion. Hyperglycemia generally resolves after successful removal of the tumor.
E. Drug or chemical-induced diabetes:

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance.

In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency.

F. Infections:

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.

G. Uncommon forms of immune-mediated diabetes:

In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD autoantibodies and approximately one-third will develop diabetes. Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues. However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. As in other states of extreme insulin resistance, patients with anti-insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance.
REVIEW OF LITERATURE

H. Other genetic syndromes sometimes associated with diabetes:

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down’s syndrome, Klinefelter’s syndrome, and Turner’s syndrome. Wolfram’s syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness.

IV. Gestational diabetes (Lawrence JM, 2008):

Gestational diabetes mellitus resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness.

It occurs in about 2% – 5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in life.

Even though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Induction may be indicated with decreased placental function. A cesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.
Table 1: Comparison of type 1 and type 2 diabetes (Harsh Mohan, 2003).

<table>
<thead>
<tr>
<th>Features</th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset</td>
<td>Usually under 40</td>
<td>Usually over 40</td>
</tr>
<tr>
<td>Frequency of occurrence</td>
<td>Less than 10%</td>
<td>Greater than 90%</td>
</tr>
<tr>
<td>HLA association</td>
<td>Linked to HLA-D</td>
<td>No association</td>
</tr>
<tr>
<td>Genetic locus</td>
<td>Unknown</td>
<td>Chromosome 6</td>
</tr>
<tr>
<td>Identical twins</td>
<td>50% Chance</td>
<td>60-80% Chance</td>
</tr>
<tr>
<td>Pathogenesis</td>
<td>Autoimmune</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Islet cell antibodies</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Exposure to environmental Triggers, (i.e., virus, Chemicals,) Endocrine diseases, Heredity etc.</td>
<td>Obesity; advancing age, family history, ethnicity; malnutrition; inactivity, high cholesterol levels.</td>
</tr>
<tr>
<td>Cause</td>
<td>Body’s inability to produce insulin</td>
<td>inability to produce enough insulin or resistance to insulin</td>
</tr>
<tr>
<td>Insulin levels &amp; Insulin resistance</td>
<td>Decreased &amp; Occasional insulin resistant</td>
<td>Variable &amp; Often insulin resistant</td>
</tr>
<tr>
<td>Common symptoms</td>
<td>Frequent urination or need to Urinate, excessive thirst, extreme hunger, fatigue, Unusual weight loss, irritability.</td>
<td>Same as type 1 symptoms and blurred vision, increased susceptibility to infection, slow to healing, gum or bladder infections.</td>
</tr>
<tr>
<td>Appearance of symptoms</td>
<td>Rapid</td>
<td>Slow</td>
</tr>
<tr>
<td>Beta-cells</td>
<td>Decreased</td>
<td>Variable</td>
</tr>
<tr>
<td>Ketoacidosis</td>
<td>Frequent</td>
<td>Rare</td>
</tr>
<tr>
<td>Complications</td>
<td>Frequent</td>
<td>Frequent</td>
</tr>
<tr>
<td>Treatment</td>
<td>Diet control, exercise, daily insulin injections.</td>
<td>Diet control, exercise, oral medication and/or insulin.</td>
</tr>
</tbody>
</table>
2.2.4. Causes of Diabetes mellitus (Ballard AM, 2000):

Type 1 diabetes mellitus:

1. Genetic predisposition.
3. Autoimmune reaction: β-cells that produce insulin in the pancreas are destroyed.

Type 2 diabetes mellitus:

1. Insulin resistance: unable to utilize insulin that the body makes because of cell receptor defect; glucose is unable to be absorbed into cells for fuel.
2. Decreased insulin secretion: pancreas does not secrete enough insulin into glucose levels.
3. Excess production of glucose from the liver is result of defective insulin secretory response; dawn phenomenons occur.

Gestational Diabetes Mellitus:

1. Insulin resistance due to pregnancy
2. Genetic predisposition.

2.2.5. Characteristics of Diabetes mellitus (Ballard AM, 2000):

Type 1 diabetes mellitus:

1. Usually occurs before 30 years of age, but can occur at any age. Peak incidence occurs during puberty, around 10-12 years of age in girls and 12-14 years in boys.
2. Abrupt onset of signs and symptoms of hyperglycemia: increased thirst and hunger, frequent urination, weight loss, and fatigue.
Type 2 diabetes mellitus:

1. Usually occur after 30 years of age, but is now occurring in children and adolescent.
2. Increased prevalence in some ethnic groups, e.g., African, Americans, Hispanic/Latino, Native Americans, Asian Americans, and Pacific Islanders.
4. Frequently obese.
5. Not prone to ketoacidosis until late in course or with prolonged hyperglycemia.
6. May or may not have symptoms of hyperglycemia.
7. May also have extreme tiredness, blurred vision, delayed healing, numbness and tingling of hands and feet, recurring yeast infection.
8. Children between the ages of 10-19 that have one or more of the following are at an increased risk:
   - Family history
   - Overweight
   - Sedentary lifestyle
   - Pre-puberty.
   - Signs of insulin resistance or conditions associated with insulin resistance hypertension, dyslipidemia, polycystic ovarian syndrome.

2.2.6. Complications of Diabetes (Haslett C, 2000):

A. Acute metabolic complications:

1. Hyperglycemia
2. Hypoglycemia
3. Acute Decompensation
   - Diabetic ketoacidosis
   - Non-ketotic hyperosmolar diabetic coma
• Lactic acidosis
• Acute circulatory failure

B. Long term complications of diabetes:

1. Microvascular
   • Eye complications – Retinopathy, Cataract
   • Diabetic Nephropathy
   • Diabetic Neuropathy
   • Foot complications – Ulceration, Infections

2. Macrovascular
   • Cardiovascular complications
   • Cerebrovascular complications
   • Peripheral arterial complication

A. Acute metabolic complication (Haslett C, 2000):

1. Hyperglycemia:

Hyperglycemia is a very common biochemical abnormality. It is frequently detected on routine biochemical analysis of asymptomatic patients, and is found during conditions which impose a burden on pancreatic b-cells, such as pregnancy, severe illness or treatment with drugs such as corticosteroids (stress hyperglycemia).

2. Hypoglycemia:

Hypoglycemia (i.e. a blood glucose < 3.5mmol/l) is a result of the treatment of diabetes rather than manifestation of the disease itself. In fact, many of patients die because hypoglycemia rather than hyperglycemia.
3. Acute decompensation:

- **Diabetic ketoacidosis:**
  Diabetic ketoacidosis is a major medical emergency and remain a serious cause of morbidity principally in people with type 1 diabetes. It is caused by insulin deficiency and an increase in catabolic hormones, leading to hepatic over-production of glucose and ketone bodies. The cardinal biochemical features of diabetic ketoacidosis are:
  - Hyperglycemia
  - Hyperketonaemia
  - Metabolic acidosis

- **Lactic acidosis:**
  **Diagnosis:**
  The diagnosis is confirmed by a high (usually >5.0 mmol/l) concentration of lactic acid in the blood.
  **Treatment:**
  Treatment is with i.v. sodium bicarbonate sufficient to raise the plasma $P^H$ to above 7.2, along with insulin and glucose.

- **Acute circulatory failure:** This can occur in any of these types of acute metabolic decompensation.

B. Long term complication of diabetes:

1. Microvascular Complications:

**Eye complication** (Hori S, 2002):

- **Retinopathy:** Microstructural and functional are two abnormalities stages before the development of retinopathy. Diabetic retinopathy is classified
into three stage based on the stage of progression of the disease, i.e.- Background, Preproliferative and Proliferative. The earliest stage is called background diabetic retinopathy and is characterized by dot or blot hemorrhages and microaneurysms associated with retinal microvascular lesions which are closely related to hyperglycemia. With the development of retinal capillary obstruction, background diabetic retinopathy progresses to the stage of preproliferative diabetic retinopathy, in which vascular zones can be detected by fluorescein angiography. In proliferative diabetic retinopathy, which develops with further progression of the disease, newly formed blood vessels, vitreous hemorrhage, and retinal detachment produce severe visual loss.

- **Cataract**: Cataract is permanent lens opacity and is the most common cause of visual deterioration in the elderly population. The lens thickens and opacifies with age and the increased metabolic insult to the lens in the people with diabetes causes these changes to accelerate and occur prematurely.

**Diabetic nephropathy** (Kikkakara R, 2001):

Nephropathy progresses through 4 stages. In stage 1 diabetic do not manifest microalbuminuria. The stage of nephropathy in which microalbuminuria is observed is referred to as “incipient nephropathy” (stage 2). Stage 3 is manifested by proteinuria and is known as “overt nephropathy”. The last stage is of renal failure which is manifested by an abnormally high serum creatinine.

**Diabetic neuropathy** (Bhadada SK, 2001):

Neuropathy, a common complication of diabetes mellitus, is generally considered to be related to duration and severity of hyperglycemia. Diabetic neuropathy has been defined as presence of symptoms and/or signs of peripheral nerve dysfunction in diabetics after exclusion of other causes, which may range from hereditary, traumatic,
compressive, metabolic, toxic, nutritional, infectious, immune mediated, neoplastic, and secondary to other systemic illnesses.

**Foot complication:**

Foot infection in patients with diabetes is among the common bacterial infections encountered in clinical practice. Unfortunately these infections and their sequelae are also the most common cause of disability and the reason for most hospital admission among diabetic patients (Shea KW, 1999).

**2. Macrovascular complications:**

**Cardiovascular complications** (Beckman JA, 2002):

- **Coronary Artery Complications (CAD):** causes much of the serious morbidity and mortality in patients with diabetes, who have a 2-4 fold increase in the risk of CAD. Diabetes also worsens early and late outcomes in acute coronary syndromes. In unstable angina pectoris or non Q wave MI compared to control, the presence of diabetes increases the risk of in-hospital MI, complications of MI and mortality.

- **Hypertension:** Hypertension (B.P $\geq$ 140-90 in mmHg) is a common comorbidity of diabetes. Hypertension is a major risk factor for cardiovascular disease and microvascular complications such as retinopathy and nephropathy. In type 1 diabetes, it is often the result of the underlying nephropathy. In type 2 diabetes, it is likely to be present as part of the metabolic syndrome i.e. obesity, hyperglycemia, dyslipidemia.

- **Cerebro vascular complications:** The risk of stroke is increased 150% to 400% for patients with diabetes, and worsening glycemic control relates directly to stroke risk.

- **Peripheral arterial diseases:** Individuals with diabetes have 2-4 fold increase in the rates of PAD. The duration of severity of diabetes can be correlated with incidence and extent of PAD. Diabetes also changes the nature of PAD. e.g. vascular calcification.
2.2.7. DIABETES MELLITUS AND FREE RADICALS:

2.2.7.1. Oxidative stress and Diabetic complications

Experimental diabetes in animals has provided considerable insight into the physiologic and biochemical derangement of the diabetic state. Many of this derangement were in the form of significant changes in lipid metabolism and structure (Sochar M, 1985). These structural changes are clearly oxidative in nature and are associated with development of vascular disease (Aynes, 1999). In diabetic rats, increased lipid peroxidation was also associated with hyperlipidemia (Morel DW, 1989). During diabetes, a profound alteration in the concentration and composition of lipids occurs. Liver and kidney are important for glucose and lipid homeostasis, they participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. Thus it is expected to have changes in liver and kidney during diabetes (Seifter S, 1982).

Several lines of evidence seem to indicate a relationship between hyperinsulinaemia and free radical production. In intact human fat cells, exposure to insulin leads to a time and dose dependent accumulation of hydrogen peroxidase in the suspension medium (Krieger Brauer H, 1992). In rat, increased insulin concentrations following intraperitoneal injection of dextrose have been found to be associated with increased free radical production (Habib MP, 1994). Since fasting hyperinsulinaemia is considered a hallmark of insulin resistance (Defronzo RA, 1991), a relationship between insulin resistance and plasma free radical concentrations cannot be excluded. The genesis of free radical concentrations in insulin resistant conditions might be due to (a) an insulin-mediated overdrive of sympathetic nervous system activity (b) a rise in plasma free fatty acid concentrations.
Many studies have shown that increased lipid peroxides and/or oxidative stress are present in diabetic subjects (Bonnefond RD and Haffner SM, 2000; Keaney JF, 1999). Oxidative stress can be increased before clinical signs of diabetic complications. However, the role of oxidative stress in the initiation and progression of diabetes remains uncertain. It is debatable whether oxidative stress precedes the appearance of diabetic complications or whether it merely reflects the presence of complications or consequence of complications. In diabetes, oxidative stress seems caused by both increased production of ROS, sharp reduction in antioxidant defenses and altered cellular redox status (West CJ, 2000). Enhanced oxidative stress in diabetes Type 2 has further a variety of important effects in atherogenesis, including lipoprotein oxidation, particularly LDL oxidation. Lipid peroxidation of polyunsaturated fatty acids, one of the radical reactions in vivo, can adequately reflect increased oxidative stress in diabetes (Slatter DA, 1999).

Diabetes-induced susceptibility to low-density lipoprotein peroxidation is prevented in the presence of vitamin E (Lie, 1996). Dietary vitamin E supplementation also improves fatty acid metabolism and decreases lipid peroxidation in tissues of diabetic rats (Celik S, 2002). Diabetes-induced changes in antioxidant enzymes in different organs are corrected to differing extents by vitamin E, but the combination of vitamin E with another antioxidant, stobadine, provides superior protection against deficits in these enzymes (Ulusu NN, 2003). As a critical antioxidant for the protection of plasma lipids, vitamins C will prooxidant conditions such as hyperglycemia (Frei B, 1990). Chronic administration of 1 gm/dl vitamin C in aged type 2 diabetes patients decreases plasma free radicals and increases cellular GSH levels over a period of 4 months (Paolisso G, 1995).
Antioxidants plays an important role in diabetes, these counter the action of free radicals by several mechanisms (Fig. 8). These mechanisms include: enzymes that degrade free radicals (Duckworth WC, 2001) proteins such as transferring that can bind metals which stimulate the production of free radicals (Abdollahi M, 2004) and antioxidants such as vitamins C and E that act as free radical scavengers (Penckofer S, 2002). In a study, the total antioxidant capacity in plasma of type 1 diabetics was shown to be 16% lower than that of normal subjects (Vessby J, 2002). Decreased activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), in the kidney of streptozocin (STZ)-induced diabetic rats has been reported (Kedziora-Kornatowska KZ, 2000). Lower risks of type 2 diabetes mellitus in individuals with higher levels of serum antioxidants, particularly those with higher levels of serum tocopherol, was also shown (Reunanen A, 1998). However, there are also studies showing no significant difference in antioxidant status between patients and healthy controls (Willems D, 1998).
The primary defense against oxidative stress in the cell rests with antioxidants, including vitamins C and E, reduced glutathione (GSH), and glutathione peroxidase (GSH-Px) (Abdollahi M, 2004). The most common antioxidant deficiencies reported in diabetes are lower levels of ascorbate, glutathione and superoxide dismutase. Lower concentrations of reduced glutathione have been documented in diabetic neutrophils and monocytes (Jialal I, 2002). It has also been observed that together with vitamin E, it can provide better control on hyperglycemia-induced oxidative stress (Fig 1.5). There are several medicinal plants the world over used in traditional medicine, which possess rich antioxidant principles and strong antioxidant activities. Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances that possess antioxidant activity (Chanwittheesuk A, 2005).

Phytochemicals with antioxidant effects include some cinnamic acids, coumarins, diterpenes, flavonoids, lignans, monoterpenes, phenylpropanoids, tannins and triterpenes (Larkins N, 2004). It has been argued that major antidiabetic activities from these plants might originate from their antioxidant principles (Carbett J, 1997). Therefore it seems that plants particularly those with high levels and strong antioxidant compounds have an important role in improvement of disorders involving oxidative stress such as diabetes mellitus. There are many investigations which have studied the effects of these plants and their antioxidant ingredients on diabetes and its complications and achieved good results. Taking the advantage of modern drugs like stobadine and its detailed mechanism of action, natural medicines may also be developed explaining their therapeutic properties and mechanism of action. These efforts may provide novel mechanism-based application of traditional medicines used in this disorder.

2.2.7.2. Reported effects of Medicinal Plants on oxidative Stress in Diabetes and its Complications:

2. Mahdi AA, 2003 has reported effect of herbal hypoglycemic agents (Allium sativum) on Oxidative stress and Antioxidant status in Diabetic rats.

3. Aloe vera leaf gel extract produces an antioxidant effect on oxidative stress in streptozotocin treated rats (Rajasekaran, 2005).

4. Pari L was demonstrated the antidiabetic effect of Boerhavia diffusa on serum and tissue lipids in experimentally induced Diabetes Mellitus.

5. Cassia auriculata L. flowers showed significant preventive effects on brain lipid peroxidation in Streptozotocin treated Diabetic rats (Latha M, 2003).

6. Ravi K, 2004 was studied the Anti-diabetic and antioxidant activity of Eugenia jambolana seed kernels on streptozotocin-induced diabetic rats.

7. Shukla R, 2004 reported antioxidant effect of aqueous extract of the bark of Ficus bengalensis in hypercholesterolaemic rabbits.


9. Bittergourd fruit juice has been used as antidiabetic and antioxidant in STZ induced diabetic state in vivo and in vitro (Sitasawad, 2000).

10. Talinum portulacifolium (Forssk) leaves have been reported to inhibit diabetes and oxidative stress in Alloxan-Induced Diabetic Rats (Rao TN, 2007).

2.2.8. Therapeutic interventions in Diabetes mellitus:

The aim of the treatment is primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and, by eliminating, various risk factors, to increase longevity. The first aim is not difficult to attain and in some elderly patients or those who lack motivation it is the only aim (Watkins PJ, 1990).
The care of diabetes on self-management is based on the patient’s clinical status and his/her ability to participate in self-care. Insulin replacement therapy is the mainstay for patients with type 1 DM while diet and lifestyle modifications are considered the cornerstone for the treatment and management of type 2 DM. Insulin is also important in type 2 DM when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications. Oral hypoglycemic agents are also useful in the treatment of type 2 DM. Oral hypoglycemic agents include sulphonylureas, biguanides, a glucosidase inhibitors and thiazolidenediones. The main objective of these drugs is to correct the underlying metabolic disorder, such as insulin resistance and inadequate insulin secretion. They should be prescribed in combination with an appropriate diet and lifestyle changes. Diet and lifestyle strategies are to reduce weight, improve glycemic control and reduce the risk of cardiovascular complications, which account for 70% to 80% of deaths among those with diabetes.

Diabetes is best controlled by either diet alone and exercise (non-pharmacological), or diet with herbal or oral hypoglycemic agents or insulin (pharmacological).

**Non-pharmacological interventions in the treatment of DM:**

It has been shown that weight reduction and an increase in daily energy expenditure decrease insulin resistance and increase glucose tolerance (Stoffers DA, 1997). In fact, advice on diet and exercise are an important part of the treatment of type 2 DM. Overweight patients are advised to restrict calorie intake, consume food with low total fat content (especially saturated fat) and high (predominately unrefined) carbohydrate content.
### Table 2: Major Nutraceuticals used for diabetes

<table>
<thead>
<tr>
<th>Nutraceuticals</th>
<th>Anti-Diabetic activity</th>
<th>Mode of action</th>
<th>Typical daily dose</th>
<th>Potential side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Lipoic acid</td>
<td>Insulin sensitizer; Anti-neuropathy</td>
<td>Anti-inflammatory (Antioxidant)</td>
<td>900-1800mg</td>
<td>GI irritation</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Insulin sensitiser; Improves inflammatory Endothelial dysfunction</td>
<td>Anti-inflammatory (Antioxidant)</td>
<td>500-2000mg</td>
<td>None reported</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Insulin sensitizer</td>
<td>Anti-inflammatory (Antioxidant)</td>
<td>600-900 mg</td>
<td>None reported</td>
</tr>
<tr>
<td>Niacin</td>
<td>Anti-hyperlipidemic</td>
<td>Anti-lipolytic; decreases rate of hepatic synthesis of VLDL and LDL</td>
<td>1000-1500mg</td>
<td>Impaired glucose tolerance; flushing</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>Improves endothelial dysfunction</td>
<td>Nitric oxide donor</td>
<td>3-6 gm</td>
<td>None reported</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Insulin sensitizer</td>
<td>Anti-inflammatory (Antioxidant)</td>
<td>100-150mg</td>
<td>GI irritation</td>
</tr>
<tr>
<td>PUFAs</td>
<td>Anti-hyperlipidemic</td>
<td>PPAR-α agonists</td>
<td>0.65-1.2gm</td>
<td>GI irritation; halitosis</td>
</tr>
<tr>
<td>Chromium</td>
<td>Glucose control</td>
<td>Enhances insulin action</td>
<td>50-400mg</td>
<td>Potential for renal toxicity (rare)</td>
</tr>
<tr>
<td>Vanadium</td>
<td>Insulin sensitizer; Glucose control</td>
<td>Insulin mimetic; Pan-tyrosine phosphate inhibitor</td>
<td>100-150mg</td>
<td>GI irritation; tissue accumulation; Uncertain long-term safety profile</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Insulin sensitizer; Glucose control</td>
<td>Not characterized Possibly enhances insulin action</td>
<td>300-400 mg; (2.5gm used for glucose control)</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Zinc</td>
<td>Glucose control</td>
<td>Anti-inflammatory (Antioxidant), Insulin binding</td>
<td>40-80 mg</td>
<td>GI irritation; metallic taste; headache</td>
</tr>
<tr>
<td>Conjugated Linoleic Acid</td>
<td>Anti-obesity</td>
<td>PPAR-α agonists</td>
<td>2-4 gm</td>
<td>GI irritation; Increased inflammation and oxidative stress</td>
</tr>
</tbody>
</table>

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The goals of therapy for type 1 or type 2 Diabetes Mellitus are to: (1) eliminate symptoms related to hyperglycemia (2) reduce or eliminate the long-term microvascular and macrovascular complications of Diabetes Mellitus, and (3) allow the patient to achieve as normal a life-style as possible. To reach these goals, the physician should identify a target level of glycemic control for each patient; provide the patient with the educational and pharmacologic resources necessary to reach this level. Not only drugs help to control hyperglycemia, but also one should take care of diet and physical activities in daily life style.

Pharmacological treatment of type-1 DM:

Because individuals with type 1 Diabetes mellitus lack endogenous insulin production, administration of basal, exogenous insulin is essential for regulating glycogen breakdown, gluconeogenesis, lipolysis, and ketogenesis. Likewise, postprandial insulin replacement should be appropriate for the carbohydrate intake and promote normal glucose utilization and storage. Various insulin preparations are given by patient to control Glucose metabolism in Body.

Insulin Preparations:

Current insulin preparations are generated by recombinant DNA technology and consist of the amino acid sequence of human insulin. Animal insulin (beef or pork) is no longer used.

Table 3: Pharmacokinetics of insulin preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Onset of action (hrs)</th>
<th>Peak effect (hrs)</th>
<th>Effective Duration (hrs)</th>
<th>Maximum Duration (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short-acting:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lispro</td>
<td>&lt;0.25</td>
<td>0.5-1.5</td>
<td>3-4</td>
<td>4-6</td>
</tr>
<tr>
<td>Regular</td>
<td>0.5-1.0</td>
<td>2-3</td>
<td>3-6</td>
<td>6-8</td>
</tr>
<tr>
<td><strong>Intermediate-acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPH</td>
<td>2-4</td>
<td>6-10</td>
<td>10-16</td>
<td>14-18</td>
</tr>
<tr>
<td>Lente</td>
<td>3-4</td>
<td>6-12</td>
<td>12-18</td>
<td>16-20</td>
</tr>
<tr>
<td><strong>Long-acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultralente</td>
<td>6-10</td>
<td>10-16</td>
<td>18-20</td>
<td>20-24</td>
</tr>
<tr>
<td>Glargine</td>
<td>4</td>
<td>3</td>
<td>24</td>
<td>&gt;24</td>
</tr>
</tbody>
</table>
Pharmacological Treatment of type -2 DM:

Pharmacologic approaches to the management of type 2 diabetes mellitus include both oral hypoglycemic agents and insulin, most physicians and patients prefer oral hypoglycemic agents as the initial choice.

**Table 4: Antidiabetic agents**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of Action</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin secretagogues</td>
<td>↑ Insulin by interacting with ATP sensitive K+channels</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>↑ Insulin by interacting with ATP sensitive K+channels</td>
<td>Lower fasting blood glucose</td>
<td>Hypoglycemia weight gain, hyperinsulinemia</td>
<td>Renal/liver disease</td>
</tr>
<tr>
<td>Meglitinide</td>
<td>Closure of ATP Dependent K+channels</td>
<td>Short onset of action, lower postprandial glucose</td>
<td>Hypoglycemia</td>
<td>Liver disease</td>
</tr>
<tr>
<td>Biguanides</td>
<td>↑ Hepatic glucose production, weight loss, ↑ glucose utilization</td>
<td>Weight loss, improved lipid profile, no hypoglycemia</td>
<td>Lactic acidosis, diarrhea, nausea, possible increased cardiovascular mortality</td>
<td>Serum creatinine &gt; 1.5mg/dl (men), &gt; 1.4mg/dl (women) radiographic contraststudies, acidosis</td>
</tr>
<tr>
<td>α-Glucosidase inhibitors</td>
<td>↑ glucose absorption</td>
<td>No risk of hypoglycemia</td>
<td>GI flatulence</td>
<td>Liver/renal disease</td>
</tr>
<tr>
<td>Thiazolidine-Diones</td>
<td>↓ Insulin Resistance, ↑ glucose utilization Act through PPAR-γ nuclear receptor</td>
<td>↓ Insulin and sulfonylurea requirements, ↓ triglycerides</td>
<td>Frequent hepatic monitoring for idiosyncratic hepatocellular injury</td>
<td>Liver disease, congestive heart failure</td>
</tr>
<tr>
<td>Medical nutrition therapy</td>
<td>↓ Insulin resistance</td>
<td>Other health benefits</td>
<td>Compliance difficult, long term success low</td>
<td>–</td>
</tr>
</tbody>
</table>

Where, ↓ Increase, ↓ Decrease
**Insulin therapy in type-2 Diabetes Mellitus:**

Modest doses of insulin are quite efficacious in controlling hyperglycemia in newly diagnosed type 2 Diabetes. Insulin should be considered as the initial therapy in type 2 Diabetes, particularly in lean individuals or those with severe weight loss, in individuals with underlying renal or hepatic disease that precludes oral glucoselowering agents, or in individuals who are hospitalized or acutely ill.

2.2.9. SCREENING MODELS FOR DIABETES (H Gerhard Vogel, 2002):

1. **Pancreatectomy in dogs:**

   The final proof for the existence of a hormone in the pancreas was furnished by Banting and Best (1922) who could reduce the elevated blood sugar levels in pancreatectomized dogs by injection of extracts of the pancreatic glands.

   **Procedure:**

   Male Beagle dogs weighing 12–16 kg are used. The animal is anesthetized with an intravenous injection of 50 mg/kg pentobarbital sodium and placed on its back. After removal of the fur and disinfection of the skin a midline incision is made from the xyphoid process reaching well below the umbilicus. Bleeding vessels are ligated and the abdomen is entered through the linea alba. The falciform ligament is carefully removed and the vessels ligated. A self-retaining retractor is applied. By passing the right hand along the stomach to the pylorus, the duodenum with the head of the pancreas is brought into the operating field. First, the mesentery at the unicate process is cut and the process itself is dissected free. The glandular tissue is peeled off from the inferior pancreatico-duodenal artery and vein. The vessels themselves are carefully preserved. Along a line of cleavage which exists between the pancreas, the pancreaticoduodenal vessels and the duodenal wall, the pancreas is separated from the duodenum and from the carefully preserved pancreaticoduodenal vessels. The small vessels to the pancreas are ligated.

   The dissection is carried out from both sides of the duodenum. In the area of the accessory pancreatic duct the glandular tissue being attached very firmly has to be
carefully removed in order to leave no residual pancreatic tissue behind. The pancreatic duct is cleaned, doubly ligated and cut between the ligatures. The dissection proceeds until one encounters a small lobe containing the main pancreatic duct. The glandular tissue adheres here firmly to the duodenum. Blunt dissection and ligation of the vessels is followed by ligation of the pancreatic duct. By pulling on the pylorus and the stomach, the pyloric and the splenic parts of the pancreas are delivered into the wound. The duodenal part is placed back into the abdominal cavity. The mesentery of the body and tail of the pancreas is cut with scissors. The small vessels are doubly ligated and cut. The pancreatic tissue is bluntly dissected from the splenic vessels.

The pancreatic branches of the splenic vessels are doubly ligated and cut. Working in direction from the spleen to the pylorus, the pyloric part of the pancreas is the last one to be dissected. Finally, all pancreatic tissue is removed. The surgical field is checked once more for pancreatic remnants. The concavity of the duodenum and its mesentery is approximated by a few silk stitches and the omentum is wrapped around the duodenum. Retroperitoneal injection of 5 ml 1% procaine solution is given to prevent intussusception of the gut. 250,000 IU penicillin G in saline solution is instilled into the peritoneal cavity. The abdominal wall and the subcutaneous layer are closed by sutures and finally the skin is sutured with continuous everting mattress stitches. After the operation, the animal receives via a jugular vein catheter for 3–4 days the following treatment 1000 ml 10% glucose solution with 10 IU human insulin Regular, 3 ml 24% sulfadiazin/trimethoprim solution, 2 ml 50% metamizol and 400 IU secretin. On the third day, the animal is offered milk.

After the animal has passed the first milk feces, it is given daily dry food together with a preparation of pancreatic enzymes. Insulin is substituted with a single daily subcutaneous dose of 34 IU Retard-Insulin. Vitamin D3 is given every three months as an intramuscular.

2. Alloxan induced diabetes:

Hyperglycemia and glucosuria after administration of alloxan has been described in several species, such as in dogs, rabbits, rats. Guinea pigs have been found to be resistant. In most species a triphasic time course is observed: an initial rise
of glucose is followed by a decrease, probably due to depletion of islets from insulin, again followed by a sustained increase of blood glucose.

**Procedure:**

Rabbits weighing 2.0 to 3.5 kg are infused via the ear vein with 150 mg/kg alloxan monohydrate (5.0 g/100 ml, pH 4.5) for 10 min resulting in 70% of the animals to become hyperglycemic and uricosuric. The rest of the animals either die or are only temporarily hyperglycemic.

Rats of Wistar or Sprague-Dawley strain weighing 150–200 gm are injected subcutaneously with 100–175 mg/kg alloxan.

Male Beagle dogs weighing 15–20 kg are injected intravenously with 60 mg/kg alloxan. Subsequently, the animals receive daily 1 000 ml 5% glucose solution with 10 IU Regular insulin for one week and canned food ad libitum. Thereafter, a single daily dose of 28 IU insulin is administered subcutaneously.

3. **Streptozotocin induced diabetes:**

The diabetogenic activity of the antibiotic streptozotocin turned out to be specifically cytotoxic to β-cells of the pancreas.

**Procedure:**

Male Wistar rats weighing 150–220 g fed with a standard diet are injected with 60 mg/kg streptozotocin intravenously. As with alloxan, three phases of blood glucose changes are observed. Initially, blood glucose is increased, reaching values of 150–200 mg% after 3 h. Six–eight h after streptozotocin, the serum insulin values are increased up to 4 times, resulting in a hypoglycemic phase which is followed by persistent hyperglycemia. Severity and onset of diabetic symptoms depend on the dose of streptozotocin. After the dose of 60 mg/kg i.v., symptoms occur already after 24–48 h with hyperglycemia up to 800 mg%, glucosuria and ketonemia.

Histologically, the β-cells are degranulated or even necrotic. A steady state is reached after 10–14 days allow the animals to use for pharmacological tests.
4. Other diabetogenic compounds:

Several other compounds have been found to induce symptoms of diabetes and/or obesity, such as dithizone, goldthioglucone, monosodium glutamate.

5. Hormone induced diabetes:

- **Growth hormone induced diabetes:** It described the diabetogenic action of pure anterior pituitary growth hormone in cats. In intact adult dogs and cats, the repeated administration of growth hormone induces an intensively diabetic condition with all symptoms of diabetes including severe ketonuria and ketonemia. Rats of any age subjected to a similar treatment do not become diabetic but grow faster and show striking hypertrophy of the pancreatic islets.

- **Corticosteroid induced diabetes:** Forced fed rats treated with cortisone shows hyperglycemia and glycosuria. In the guinea pig and in the rabbit, experimental corticoid diabetes could be obtained without forced feeding in the rat, the adrenal cortex, stimulated by corticotrophin, has the capacity to secrete amounts of steroids which induce steroid diabetes.

6. Insulin deficiency due to insulin antibodies:

A transient diabetic syndrome can be induced by injection of guinea pig antiinsulin serum in various species.

**Procedure:**

Bovine insulin, dissolved in acidified water (pH 3.0), is incorporated in a water-oil emulsion based on complete Freund’s adjuvant or a mixture of paraffin oil and lanolin. A dose of 1 mg insulin is injected in divided doses subcutaneously to male guinea pigs weighing 300–400 g. Injections are given at monthly intervals and the guinea pigs are bled by cardiac puncture two weeks after the second and subsequent doses of antigen. It is possible to get 10 ml blood from every animal once a month. Intravenous injection of 0.25–1.0 ml guinea pig anti-insulin serum to rats induces a dose-dependent increase of blood glucose reaching values up to 300 mg%.
This effect is unique to guinea pig anti insulin serum and is due to neutralization by insulin antibodies of endogenous insulin secreted by the injected animal. In this way a state of insulin deficiency is induced. It persists as long as antibodies capable of reacting with insulin remain in the circulation. Slow rate intravenous infusion or intraperitoneal injection prolongs the effect for more than a few hours. However, large doses and prolonged administration accompanied by ketonemia, ketonuria, glucosuria, and acidosis are fatal to the animals. After lower doses, the diabetic syndrome is reversible after a few hours.

7. Virus induced diabetes:

Type I diabetes mellitus may be due to virus infections and β-cell specific autoimmunity. The D-variant of encephalomyocarditis virus selectively infects and destroys pancreatic β-cells in susceptible mouse strains similar to human insulin-independent diabetes. Adult male ICR Swiss mice are susceptible to the diabetogenic effect of the D-variant of encephalomyocarditis virus in contrast to adult C3H/HeJ male mice which are relatively resistant. Pretreatment with cyclosporin A, a potent immunosuppressive drug, results in increased severity and incidence of diabetes in susceptible ICR Swiss mice and induction of diabetes in resistant C3H/HeJ mice.

Intoxicant used in the study:

Alloxan:

Formula: C₄H₂N₂O₄
Molar mass: 142.07 g/mol
Melting point: 256 °C

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig (Bhattacharya S, 2012).
Alloxan is structurally similar to glucose, hydrophilic (requires GLUT2 for beta-cell uptake), unstable, and has a short biologic half-life of 1.5 minutes at normal body temperature which can be extended with lower temperatures (Lenzen, 2008).

Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus. The mechanism of alloxan action has been thoroughly studied which currently can be characterized quite well. Several experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment (Szkudelski T, 1998; Lachin T, 2012). This particular alloxan-induced insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used (Kliber A, 1996). Further, the alloxan action in the pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity.

Moreover, in pancreatic beta cells, the reduction process occurs in the presence of different reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups (Lenzen S, 1991; Zhang H, 1992). Alloxan reacts with two -SH groups in the sugar binding site of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. As a result of alloxan reduction, dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle for the generation of reactive oxygen species (ROS) and superoxide radicals (Munday R, 1988; Das J, 2012). The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions (Sakurai K, 1995). In addition, superoxide radicals undergo dismutation to yield hydrogen peroxide (H2O2) in the presence of superoxide dismutase. As a result, highly reactive hydroxyl radicals are formed according to the Fenton reaction in the presence of ferrous and H2O2.

Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. The fragmentation of DNA takes place in the beta cells exposed to alloxan that causes DNA damage, which stimulates poly ADP-ribosylation, a process participating in DNA repair. Antioxidants like superoxide dismutase, catalase and the
non-enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity (Ebelt H, 2000). In addition, the disturbance in intracellular calcium homeostasis has also been reported to constitute an important step in the diabetogenic action of alloxan. It has been noted that alloxan elevates cytosolic free Ca$_{2+}$ concentration in the beta cells of pancreatic islets (Park BH, 1995). The calcium influx is resulted from the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca$_{2+}$ ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets (Etuk EU, 2010; Szkudelski T, 2010; Lenzen S, 2008).
2.3. INTRODUCTION TO HEPATOTOXICITY

Liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturb body metabolism. In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus (Patel RB, 1998). The agent should protect against such damage, especially of one which facilitates regeneration by proliferation of parenchymal cells after damage and arrest growth of fibrous tissue (Roge Dahanukar S et al., 1984).

There is no remedy for liver diseases, which are so prevalent in the population. The treatment is mainly symptomatic (Roge N Dahanukar S et al., 1984). Scientists and some industrialists deliberated on various prospective plant remedies for ailments of liver disorder management. In the decade 70s, the world scientific community concentrated on a herbal plant Vinca rosa.

Then in 80s the attention was focused on Panax ginseng. Now, the news of multifarious activities of the Neem tree indicates that it may become centre for research in 90s. Indian Council of Medical Research, New Delhi, in its revived research on traditional medicine, had adopted liver diseases as one among six thrust areas and for multidisciplinary study.

Screening of activeconstituents from Kutki (Picrorhiza Kurroa), Bhoomyamalaki (Phyllanthus niruri) have shown marked protection against jaundice. Hepatitis continues to be a major health problem in urban areas in India, and several studies in viral hepatitis were under investigation by the ICMR; for example, extracts of milk thistle (Silybum marianum) fruits under investigation for the treatment of alcoholic hepatitis. According to Indian Society of Gastroenterology, Mulethi (Glycyrrhiza glabra) prevents multiplication of viruses inside liver cells. The disorder of liver may be acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory liver diseases) and liver cirrhosis (fibrosis of the liver). Liver enzymes act as an index of sub-clinical hepatic damage. Serum glutamic pyruvic transaminase (SGPT), serum glutamicoxaloacetic pyruvic transaminase (SGOT),
Serum lactic dehydrogenase (LDH) and Serum alkaline phosphatase are reported as an index of hepatic injury and cholestasis (Doreswamy R, 1995).

2.3.1. LIVER (ANATOMY, HISTOLOGY & PHYSIOLOGY)

The liver is the heaviest gland of the body, weighing about 1.4 kg in an average adult.

2.3.1.1. Anatomy of liver (Harsh Mohan, 2005):

Anatomy liver is the largest organ in the body weighing 1400-1600 gm in the males and 1200-1400 gm in females. There are 2 main anatomical lobes-right and left. The right being about six times the size of the left lobe. The right lobe has quadrate lobe on its inferior surface and a caudate lobe on the posterior surface. The right and left lobes are separated anteriorly by a fold of peritoneum called the falciform ligament, inferiorly by the fissure for the ligamentum teres and posteriorly by the fissure for the ligamentum venosum.

The porta hepatis is the region on the inferior surface of the right lobe where blood vessels, lymphatic and common hepatic duct forms the hilum of the liver. A firm smooth layer of connective tissue called Glisson’s capsule encloses the liver and is continuous with the connective tissue of the porta hepatis forming a sheath around the structures in the porta hepatis. The liver has a double blood supply, the portal vein brings the venous blood from the intestine and spleen and the hepatic artery coming from the coeliac axis supplies arterial blood to the liver. This dual blood supply provides sufficient protection against infarction in the liver. The portal vein and hepatic artery divide into branches to the right and left lobes in the porta. The right and left hepatic ducts also join in the porta to form the common hepatic duct. The venous drainage from the liver is into the right and left hepatic veins which enter the inferior vena cava. Lymphatics and the nerve fibres accompany the hepatic artery into their branching and terminate around the porta hepatis.
2.3.1.2. Histology

The hepatic parenchyma is composed of numerous hexagonal or pyramidal classical lobules each with a diameter of 0.5 to 2 nm. Each classical lobule has a central tributary from the hepatic vein and at the periphery are 4 to 5 portal tracts or triads containing branches of bile duct, portal vein and hepatic artery. Cords of hepatocytes and bloodcontaining sinusoids radiate from the central vein to the peripheral portal triads. The functioning lobule or liver acinus as described by Rappaport has a portal triad in the centre and is surrounded at the periphery by portions of several classical lobules.

However in most descriptions on pathology of the liver, the term lobule is used in its classical form.

The blood supply to the liver parenchyma flows to the portal triads and to the central veins. Accordingly, the hepatic parenchyma of liver lobule is divided into 3 zones.

- Zone – 1 or the periportal (peripheral) area is closed to the arterial and portal blood supply and hence bears the brunt of all forms of toxic injury.
- Zone – 3 or the centrilobular area surrounds the central vein and is most remote from the blood supply and thus suffers from the effects of hypoxic injury.
- Zone – 2 is the intermediate midzonal area.

The hepatocytes are polygonal cells with a round single nucleus and a prominent nucleolus. The liver cells have a remarkable capability to undergo mitosis and regeneration. Thus it is not uncommon to find liver cells containing more than one nuclei and having polyploidy up to octoploidy. A hepatocyte has 3 surfaces; one facing the sinusoid and space of disse, the second facing the canaliculus and the third facing neighbouring hepatocytes.

The blood-containing sinusoids between cords of hepatocytes are lined by discontinuous endothelial cells and scattered flat Kupffer cells belonging to the reticuloendothelial system.
The space of disse is the space between hepatocytes and sinusoidal lining endothelial cells. A few scattered fat storing cells lie within the space of disse.

The portal triad or tract besides containing portal vein radical, the hepatic arteriole and bile duct has a few mononuclear cells and a little connective tissue considered to be extension of Glisson’s capsule. A limiting plate of hepatocytes surrounds the portal triads.

![Liver Lobule](image)

**Figure 8: Schematic drawing of a liver lobule, the functional unit of the liver, with its periportal and perivenous regions (Cunningham and Van Horn 2003).**

The intrahepatic biliary system begins with the bile canaliculi interposed between the adjacent hepatocytes. The bile canaliculi are simply grooves between the contact surfaces of the liver cells and are conveved by microvilli. These canaliculi join at the periphery of the lobule to drain eventually into terminal bile ducts or ductules (canal of hering) which are lined by cuboidal epithelium.
Blood supply of the liver:

The liver receives blood from two sources. From the hepatic artery it obtains oxygenated blood, and from the hepatic portal vein it receives deoxygenated blood containing newly absorbed nutrients, drugs, and possibly microbes and toxins from the gastrointestinal tract (Fig. 8).

2.3.1.3. Functions of the liver:

Besides secreting bile, which is needed for absorption of dietary fats, the liver performs many other vital functions:

1. Carbohydrate metabolism:
   
   The liver is especially important in maintaining a normal blood glucose level. When blood glucose level is low, the liver can break down glycogen to glucose and release glucose into the bloodstream. The liver can also convert amino acids into lactic acid and galactose into glucose. When blood glucose is high, as occurs just after eating a meal, the liver converts glucose to glycogen and triglycerides for storage.

2. Lipid metabolism:

   Hepatocytes store some triglycerides, break down fatty acids to generate ATP, synthesize lipoproteins, which transport fatty acids, triglycerides and cholesterol to and from body cells, synthesize cholesterol and use cholesterol to make bile salts.

3. Protein metabolism:

   Hepatocytes deaminate (remove the amino group, NH2 from) amino acids so that the amino acids can be used for ATP production or converted to carbohydrate or fats. The resulting toxic ammonium is then converted into the much less toxic urea, which is excreted in urine. Hepatocytes also synthesize most plasma proteins, such as alpha and beta globulins, albumin, prothrombin and fibrinogen.
4. Excretion of bilirubin:

Bilirubin, derived from the blood cells is absorbed by the liver from the blood and secreted into bile. Most of the bilirubin in bile is metabolized in the small intestine by bacteria and eliminated in feces.

5. Synthesis of bile salts:

Bile salts are used in the small intestine for the emulsification and absorption of lipids, cholesterol, phospholipids and lipoproteins.

Storage: in addition to glycogen, the liver is a prime storage site for certain vitamins (A, B12, D, E, and K) and minerals (iron and copper), which are released from the liver when needed elsewhere in the body.

6. Phagocytosis:

The stellate reticuloendothelial (kupffer’s) cells of the liver phagocytes aged red blood cells and white blood cells and some bacteria (Tortora, 2000).

7. Processing of drug and hormones:

The liver can detoxify substances such as alcohol or excrete drugs such as penicillin, erythromycin, and sulfonamides into bile. It can also chemically alter or excrete thyroid hormones and steroid hormones such as estrogen and aldosterone.

Functions of hepatocytes:

The liver serves both as an exocrine and a gland. The exocrine secretion of the liver is bile. The critical factor in bile formation is the secretion of bile acids and bile salts, detergents important for the emulsification of fat in the intestine. Bile also has an excretory function. Conjugates, both of endogenous materials such as bilirubin and some xenobiotics are secreted into bile.

In its second role as an endocrine gland, the liver secretes almost all the major proteins of plasma with the exception of the immunoglobulins. In addition the
liver plays a central role in lipid metabolism, taking up chylomicrons arriving in the blood from the intestine and re-packaging their lipid with a new group of proteins to form very low density lipoprotein (VLDL) particles which are then exported from the liver. The transport of proteins through the exocytic pathway is especially sensitive to changes in intracellular ATP. This is reflected in the accumulation of fat in the damaged liver and fall in plasma proteins and disturbances in blood clotting that follow long standing liver damage.

In addition to forming the bulk of the plasma proteins, the liver is also responsible for their recycling. The liver is also responsible for recycling old red blood cells. Principally Kupffer cells carry out this task.

In addition to these roles the liver acts as the centre of intermediary metabolism in the body and the liver stores of sugar, in the form of glycogen. Further it is also major site for the conversion of sugars to lipids and for conversion of amino acids to sugars and lipids.

This may have marked consequences. For example in rats the metabolic demands of lactation result in a 30 percent increase in liver weight, exacerbating the toxicity of liver enlarging agents such as BHT. In addition the liver is main or in the case of humans almost the sole, site of de novo cholesterol synthesis. The result is that liver damage causes marked disturbances in endocrine function, for example liver cirrhosis results in feminization in men and masculinization in women.

One more important function of the liver is metabolism and excretion of variety of hydrophobic compounds. The liver metabolizes and excretes both endogenous body constituents such as haem or steroids, nutrient and exogenously administered xenobiotics, toxins, etc. In general the physiological role of the metabolism is to prepare the compounds for excretion. The metabolic reactions are conveniently divided into two phases. Phase I reactions involve chemical modification of the reactant normally by oxidation whereas phase II reactions are biosynthetic, generally involving conjugation with a hydrophilic moiety such as glucoronic acid or glutathione. Under most circumstances these biphasic metabolic reactions are protective and results in the removal of potentially harmful materials
from the body. However, under some circumstances the metabolites are markedly more toxic / potent than the parent compound.

Hepatic tissue or liver is involved in the metabolic degradation of various endogenous and exogenous substances, resulting in the generation of various intermediary highly reactive species. Hence it is susceptible to the attack by highly reactive species generated during the metabolic functioning of the liver, resulting in the hepatic damage. Various types of hepatic diseases inducted by xenobiotics, toxins, etc are given as below

2.3.2. Diseases of liver:

The liver may take several forms and involve the hepatocytes, vascular cells or bile ducts.

The most important diseases are (Damjanov I, 1996; Gennavo AR, 2000)–

1. Biliary obstruction.
2. Metabolic lesions caused by genetic disease or exogenous substance, such as alcohol.
3. Inflammation especially caused by hepatitis viruses.
4. Cirrhosis.
5. Neoplasia.

**Biliary obstruction:**

Bile flow obstruction results to jaundice. Lesions in the main extra hepatic bile duct, such as carcinoma, impacted bile stones, or sclerosing cholangitis typically cause obstructive jaundice. Prolonged bile duct obstruction may cause secondary biliary cirrhosis.

**Metabolic disorders:**

Metabolic disorder of the liver may be hereditary (genetic) or acquired. Representative hereditary rubinemias and disorders involving intermediate metabolism of lipids, carbohydrates, proteins and heavy metals.
**Congenital metabolic disorder:**

Congenital hyperbilirubinemia occurs in several forms. The best known congenital jaundice syndromes are Gilbert syndrome, Rotor syndrome, and Dubin-Johnson syndrome.

Genetic enzyme deficiencies such as alpha-1-antitrypsin deficiency may also result in liver injury, which ultimately lead to cirrhosis.

**Acquired metabolic disorders:**

Metabolic disorder can be induced in liver cells by a variety of ingested substances such as toxins, drugs, foods and beverages. Alcohol produces three types of liver disease such as hepatomegaly, alcoholic hepatitis and cirrhosis.

**2.3.3. Viral hepatitis:**

Acute viral hepatatis is a systemic infection manifested primarily by an acute attack on the hepatocytes. Five hepatotropic viruses have been identified (HAV, HBV, HCV, HDV, HEV). Hepatitis A (HAV) causes acute self-limited disease that is transmitted orally. Hepatitis B (HBV) and Hepatitis C viruses (HCV) are transmitted by the exchange of body fluids such as through blood transfusion or sexual contacts. Hepatitis D viruses (HDV) are a viroid that causes inflammation only in concrete with HBV. Hepatitis E viruses (HEV) is transmitted by enteric route and cause self limited diseases.

Chronic hepatitis is an uncommon, but important, complication of HBV and combined HBV – HDV infection. The liver injury results from inflammatory immune attack against hepatocytes. In drug induced hepatitis, a number of drugs have been reported, including methyldopa, nitrofurantoin, isoniazide, ketonazole, and acetaminophen.

**2.3.4. Cirrhosis:**

Cirrhosis is a chronic liver disease characterized by wide spread fibrosis and regenerative nodules, which diffusely replace the normal liver parenchyma
The major causes of cirrhosis are alcoholism and viral hepatatis (HBV, HCV, and HDV).

2.3.5. Liver tumor:

Primarily liver tumor may originate from liver cells, from bile ductules and less often from Kupffer cells and connective tissue cells of hepatic capsule and portal tracts.

Hepatocellular carcinoma (malignant hepatonia) is the most common primary malignant liver tumor. Cholangio cellular carcinoma is a malignant tumor of bile ducts.

Table 5: Clinical consequences of liver disease

<table>
<thead>
<tr>
<th>Characteristics signs</th>
<th>Hepatic dysfunction</th>
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<tbody>
<tr>
<td></td>
<td>Jaundice and cholestasis</td>
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<tr>
<td></td>
<td>Hypoalbuminemia</td>
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<tr>
<td></td>
<td>Hyperammonemia</td>
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<tr>
<td></td>
<td>Hypoglycemia</td>
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<tr>
<td></td>
<td>Fetor hepaticus</td>
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<tr>
<td>Palmar erythema</td>
<td>Spider angiomas</td>
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<td></td>
<td>Hypogonadism</td>
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<td></td>
<td>Gynecomastia</td>
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<td></td>
<td>Weight loss</td>
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<td></td>
<td>Muscle wasting</td>
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<tr>
<td></td>
<td>Portal hypertension from cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Ascites</td>
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<tr>
<td></td>
<td>Splenomegaly</td>
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<tr>
<td></td>
<td>Hemorrhoids</td>
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<tr>
<td></td>
<td>Caput medusae-abdominal skin</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Life-threatening complications</th>
<th>Hepatic failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple organ failure</td>
</tr>
<tr>
<td></td>
<td>Coagulopathy</td>
</tr>
<tr>
<td></td>
<td>Hepatic encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Hepatorenal syndrome</td>
</tr>
<tr>
<td></td>
<td>Portal hypertension from cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Esophageal varices, risk of rupture</td>
</tr>
<tr>
<td>Malignancy with chronic disease</td>
<td>Hepatocellular carcinoma</td>
</tr>
</tbody>
</table>
### Table 6: Classification of Hepato-toxic substances (Zimmerman HJ, 1978):

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inorganic agents</strong></td>
<td>Metals and metalloids: antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc, hydrazine, derivative, iodides.</td>
</tr>
<tr>
<td><strong>Organic agents</strong></td>
<td></td>
</tr>
<tr>
<td>Natural: Plant toxins</td>
<td>Albitocin, cycasin, nutmeg, tannic acid, icterogenin, pyrrolidizines, safe role, indospicine.</td>
</tr>
<tr>
<td><strong>Mycotoxins</strong></td>
<td>Aflatoxins, cyclochlorrotine, ethanol, luteoskyrin, griseofulvin, sporidesmin, tetracycline and other antibiotics.</td>
</tr>
<tr>
<td><strong>Bacterial toxins</strong></td>
<td>Exotoxins (C. diphtheria, Clostridium, botulinus) endotoxins, ethionine.</td>
</tr>
<tr>
<td><strong>Synthetic Non-medicinal agents</strong></td>
<td>Haloalkanes and haloolephins, Nitroalkanes, Chloroaromatic compounds. Nitroaromatic compound, organic amines, Azo compounds phenol and derivatives, various other organic compounds.</td>
</tr>
</tbody>
</table>

### Table 7: Medicinal agents

<table>
<thead>
<tr>
<th>Category of drugs</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Neuro psychotropics</td>
<td>Hydrazine, tranylcyromine Anticonvulsants, antidepressants.</td>
</tr>
<tr>
<td>2. Anti-inflammatory and antimuscle spasm agents</td>
<td>Cinchopen, chlochicine, ibuprofen, salicylates, indomethacin.</td>
</tr>
<tr>
<td>3. Hormonal derivatives and other drugs used in endocrine disease</td>
<td>Acetohexamide, Azepinamide, Carbutamide, Tolbutamide.</td>
</tr>
<tr>
<td>4. Antimicrobials</td>
<td>Clindamycin, novobiocin, penicillin, tetracycline, sulfonamide, amodiaquine, isoniazid, rifampin</td>
</tr>
<tr>
<td>5. Antineoplastic</td>
<td>L-Asparaginase, azacytidine, methotrexate, 6-mercaptopurine, chlorambucil, clavicin.</td>
</tr>
</tbody>
</table>
Table 8: Clinically important Hepatotoxins and their mechanism in causing hepatotoxicity (Zimmerman HJ, 2002; Ishak KG, 1982)

<table>
<thead>
<tr>
<th>Category of agents</th>
<th>Mechanism of action</th>
<th>Histologic lesion</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>Direct Physiochemical destruction by peroxidation of hepatocytes.</td>
<td>Necrosis and/or steatosis</td>
<td>CCl₄, phosphorous</td>
</tr>
<tr>
<td>Indirect cytotoxic</td>
<td>Interference with hepatocellular metabolic pathways</td>
<td>Steatosis or necrosis</td>
<td>Ethionine, ethyl alcohol, tetracycline</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Interference with bile excretory pathways</td>
<td>Cholestasis duel destruction</td>
<td>Methylene anabolic dianiline, and contraceptive steroids.</td>
</tr>
<tr>
<td>Host idiosyncracy</td>
<td>Drug allergy</td>
<td>Necrosis or cholestasis</td>
<td>Chlorpromazine, phenytoin, sulfonamides.</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Production of hepatotoxic metabolites</td>
<td>Necrosis or cholestasis</td>
<td>Isoniazid, valproic acid</td>
</tr>
</tbody>
</table>

2.3.6. SCREENING MODELS FOR HEPATOPROTECTIVE ACTIVITY

In general, the therapeutic values of drugs are evaluated in animals experimentally made sick. Detailed efficacy and toxicity studies in experimental animals should be followed by clinical trials. Detailed biochemical and other in vitro assays are required to determine the mechanism of action. Both in vivo and in vitro test systems are used to assess hepatoprotective activity of herbal drugs.

However, a single and simple screening method is not available to identify hepatoprotective drugs with confidence.
1. In-vivo models:

a) Toxic chemicals-induced liver damage

b) Reduction in CCl₄-induced prolongation of Hexobarbitone-induced sleeping time

c) Anti-hepatitis virus activity

d) Choleretic activity

e) Regeneration of hepatocytes

2. In-vivo models:

a) Toxic chemicals-induced liver damage (Chrungo VJ, 1997):

A toxic dose or repeated doses of a known hepatotoxin (carbon tetrachloride (CCl₄), paracetamol, thioacetamide, alcohol, D-galactosamine, allylalcohol, etc.) is administered, to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. If the hepatotoxicity is prevented or reduced the test substance is effective.

Liver damage and recovery from damage are assessed by measuring serum marker enzymes, bilirubin, histopathological changes in the liver, biochemical changes in liver (e.g. hydroxyproline, lipid, etc.) and bile flow.

When liver is damaged liver enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase enter into the circulation. An increase in the levels of these marker enzymes in the serum is
an indication of liver damage. Other effects of induced liver damage such as reduction of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromsulphthalein can be used in the evaluation of hepatoprotective plants.

The hepatoprotective effect of a drug against different hepatotoxins differs especially when the mechanisms of action of the toxins are different (Curtis D, 2001). Therefore, the efficacy of each drug has to be tested against hepatotoxins which act by different methods.

b) Reduction in CCl₄-induced prolongation of hexobarbitone - induced sleeping time (Chaudhury SK, 1978):

This method is used to screen anti-CCl₄ toxicity of drugs in animals. Hepatotoxic chemicals like CCl₄ reduce the levels of drug metabolizing enzyme in liver. Therefore metabolism of hexabarbitone is reduced resulting in prolongation of hexabarbitone induced sleeping time. If a plant drug reduces this CCl₄-induced prolongation of 'sleeping time', the drug can be considered hepatoprotective against CCl₄ toxicity (Care has to be taken to see that the drug has no direct effect on drug metabolising enzymes or necrosis).

Initially, carbon-halogen bond is cleaved by cytochrome P-450 (CYP2E1) to form chloride anion and trichloromethyl radical (*CCl₃).

This radical reacts with oxygen to give a peroxy radical i.e., Trichloromethylperoxy radical. Probably small quantities of CO may appear to be generated through dichlorocarbene intermediates.

\[
\text{CCl}_3^* + \text{O}_2 \rightarrow \text{CCl}_3\text{O}_2
\]

On the whole, CCl₄-carbon is covalently bound to microsomal lipids and proteins and subsequent covalent binding to cellular macromolecules. Its hepatoprotective action begins with change in endoplasmic reticulum, which results in loss of metabolic enzymes located in the intracellular structure.
c) **Anti-hepatitis virus activity** (Freiman JS, 1988):

At present, simple in vivo test systems are not available to determine antihepatitis virus activity in rodent models. However, duck and monkey models have been introduced to test antihepatitis B activity. This area needs to be strengthened.

d) **Choleretic activity** (Kervar SS, 1976):

Techniques are available to collect bile by cannulating the bile duct, in anaesthetized as well as conscious animals, to study the effect of drugs on their secretion.

e) **Regeneration of hepatocytes** (Kervar SS, 1976):

The effect of a drug on hepatocyte regeneration can be tested by surgical removal of a portion of the liver in experimental animals. (Primary culture of hepatocytes may be used to study the effect of the drug on hepatocyte multiplication in-vitro).

2. **In- vitro studies** (Bishayee A, 1995):

Fresh hepatocytes preparations and primary cultured hepatocytes are used to study direct antihepatototoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the plant drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An increase in the activities in the medium indicates liver damage. Parameters such as hepatocytes multiplication, morphology, macromolecular synthesis and oxygen consumption are determined. Effective antiviral assays using cell culture and PCR techniques remain to be developed.

a) **Biochemical assays** (Ahmed S, 2000):

Since, many toxic chemicals induce liver damage by inducing lipid peroxidation and/or oxidative damage to DNA and reduction in the levels of glutathione, assessment of antioxidant property is useful. Antioxidant property of plant drugs is studied using liver homogenates, isolated liver cell membranes, DNA, etc. In the process leading to cirrhosis, accumulation of connective tissue and parenchymal regeneration are competing events. Therefore, the search for agents to prevent liver
cirrhosis is, also focused on inhibitors of excessive connective tissue formation in the liver. Fibrosuppressive effects by inhibitors of protein hydroxylation can be screened. (The desired organ specificity has to be tested in models of liver cirrhosis and fibrosis in-vivo).

b) Dietary prevention of liver damage (Ki-Tae Ha, 2004):

Dietary modifications can, to a large extent, prevent toxic environmental chemicals-induced liver damage. Most of the hepatotoxins damage liver directly or indirectly by oxidative damages. Antioxidant containing vegetables (e.g. carrot.), spices such as turmeric, vitamin E, etc, when included in the diet, can protect liver from damage caused by oxidative mechanisms of toxic chemicals. It has been shown that carrot juice can protect mice from CCl4-induced hepatotoxicity. In-vitro experiments have demonstrated strong anti hepatotoxic action of the curcuminoids present in turmeric (Hostettman K, 1987).

Intoxicant used in the study:

Carbon Tetrachloride (CCL4):

Formula: CCl₄
IUPAC ID: Tetrachloromethane
Boiling point: 76.72 °C
Molar mass: 153.82 g/mol
Soluble in: Properties of water

Liver injury due to oral administration of carbontetrachloride was first reported by Cameron and Karunaratne, 1936. Since then this method of induction of hepatotoxicity has been widely and successfully used and reported by numerous investigators.
Mechanism of carbon tetrachloride induced hepatic damage

CCl₄ is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury (Chungoo V, 1997).

CCl₄ induced fatty liver and cell necrosis and play a significant role in inducing triacylglycerol accumulation, depletion of GSH, increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzyme activity. Being cytoplasmic in location the damage marker enzymes GOT, GPT and HDL are released in the serum (Ji-TH, 2004).

It is now generally accepted that the hepatotoxicity of CCl₄ is the result of reductive dehalogenation, which is catalyzed by cytochrome P450 enzyme and forms the highly reactive trichloromethyl free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical.

The free radical can form covalent bond with sulfahydryl group, such as glutathione (GSH), protein thiol and lipids or abstracting a hydrogen atom from an unsaturated lipid. This covalent binding of free radical to cell macromolecules is considered the initial step in a chain of events, which eventually leads to membrane lipid peroxidation, liver damage and finally cell necrosis (Ahmed S, 2000; Lee KJ et al, 2004; Hye GJ, 1999; Wang BJ, 2004).

CCl₄ is reductively converted by P₄₅₀ to the trichloromethyl radical the fate of this radical is of interest. First the radical add covalently to unsaturated fatty acids, trichloromethyl fatty acids, particularly of membrane phospholipids.
Recently these substituted fatty acids have been noted to be partially resistant to replace from endoplasmic reticular phospholipase A2.

This seems to be result of cross linking of trichloromethyl fatty acid radical which adds to double bond of another adjacent fatty acids (Fig. 10).

The physiologic significance of this cross-linking on membrane structure and function may be great importance, particularly if these phospholipids are transformed to other critical sites in the cell. Besides covalent binding to lipid, the cells can abstract an
electron from unsaturated fatty acids, yielding CHCl₃ and or fatty acid radical. Either the trichloromethyl fatty acid radical or the fatty acid radical can react with oxygen to form peroxy radical, which initiates the lipid peroxidation chain reaction (Vlacheva K, 2004).

Table 9: Plants reported as hepatoprotective agents

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of plant</th>
<th>Part of plant</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cistus laurifolius</td>
<td>Leaf</td>
<td>Cistaceae</td>
</tr>
<tr>
<td>2</td>
<td>Beta vulgaris</td>
<td>Root</td>
<td>Chenopodiaceae</td>
</tr>
<tr>
<td>3</td>
<td>Chelidonium majus and Myrica cerifera</td>
<td>Whole plant</td>
<td>Papaveraceae</td>
</tr>
<tr>
<td>4</td>
<td>Pterocarpus santalinus</td>
<td>Stem</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>5</td>
<td>Psidium guajava</td>
<td>Leaf</td>
<td>Myrtaceae</td>
</tr>
<tr>
<td>6</td>
<td>Azadirachta indica</td>
<td>Leaf</td>
<td>Meliaceae</td>
</tr>
<tr>
<td>7</td>
<td>Balanites aegyptiaca</td>
<td>Bark</td>
<td>Simaroubaceae</td>
</tr>
<tr>
<td>8</td>
<td>Diospyros cordifolia</td>
<td>Bark</td>
<td>Ebenaceae</td>
</tr>
<tr>
<td>9</td>
<td>Lactuca scariola</td>
<td>Leaf</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>10</td>
<td>Calotropis procera</td>
<td>Flower</td>
<td>Asclepiadaceae</td>
</tr>
<tr>
<td>11</td>
<td>Ficus lacor</td>
<td>Bark</td>
<td>Moraceae</td>
</tr>
<tr>
<td>11</td>
<td>Hemidesmus indicus</td>
<td>Root</td>
<td>Asclepiadaceae</td>
</tr>
<tr>
<td>12</td>
<td>Cichorium intybus</td>
<td>Bark</td>
<td>Cistaceae</td>
</tr>
<tr>
<td>13</td>
<td>Cichorium intybus</td>
<td>Root</td>
<td>Compositae</td>
</tr>
<tr>
<td>14</td>
<td>Berberis tinctoria</td>
<td>Leaf</td>
<td>Berberidaceae</td>
</tr>
<tr>
<td>15</td>
<td>Coccinia grandis</td>
<td>Fruit</td>
<td>Cucurbitaceae</td>
</tr>
<tr>
<td>16</td>
<td>Solanum torvum</td>
<td>Fruit</td>
<td>Solanaceae</td>
</tr>
<tr>
<td>17</td>
<td>Pterocarpus marsupium</td>
<td>Bark</td>
<td>Papilionaceae</td>
</tr>
<tr>
<td>18</td>
<td>Cassia fistula</td>
<td>Leaf</td>
<td>Caesalpinaceae</td>
</tr>
<tr>
<td>19</td>
<td>Abutilon indicum</td>
<td>Leaf</td>
<td>Malvaceae</td>
</tr>
<tr>
<td>20</td>
<td>Leucas hirta</td>
<td>Leaf</td>
<td>Labiateae</td>
</tr>
</tbody>
</table>
2.4. DEPRESSION

Depression is the most common of the affective disorders (defined as disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions.

Worldwide, depression is a major cause of disability and premature death. In addition to the significant suicide risk, depressed individuals are more likely to die from other causes, such as heart disease or cancer (Rang HP, 2003).

2.4.1.1. The symptoms of depression include emotional and biological component:

Emotional symptoms:

- Misery, apathy and pessimism
- Low self-esteem: feelings of guilt, inadequacy and ugliness
- Indecisiveness, loss of motivation.

Biological symptoms:

- Retardation of thought and action
- Loss of libido
- Sleep disturbance and loss of appetite

There are two distinct types of depressive syndrome, namely unipolar depression, in which the mood swings are always in the same direction, and bipolar affective disorder, in which depression alternates with mania. Mania is in most respects exactly the opposite, with excessive exuberance, enthusiasm and self-confidence, accompanied by impulsive actions, these signs often being combined with irritability, impatience and aggression, and sometimes with grandiose delusions of the Napoleonic kind. As with depression, the mood and actions are inappropriate to the circumstances (Rang HP, 2003).
2.4.1.2. Unipolar depression:

It is commonly (about 75% of cases) non-familial, clearly associated with stressful life events, and accompanied by symptoms of anxiety and agitation; this type is sometimes termed reactive depression. Other cases (about 25%, sometimes termed endogenous depression) show a familial pattern, unrelated to external stresses, and with a somewhat different symptomatology. This distinction is made clinically, but there is little evidence that antidepressant drugs show significant selectivity between these conditions.

2.4.1.3. Bipolar depression:

It usually appears in early adult life, is less common and results in oscillating depression and mania over a period of a few weeks. There is a strong hereditary tendency, but no specific susceptibility genes have been identified either by genetic linkage studies of affected families, or by comparison of affected and non-affected individuals.

2.4.2. The monoamine theory of depression:

The main biochemical theory of depression is the monoamine hypothesis, proposed by Schildkraut in 1965, which states that depression is caused by a functional deficit of monoamine transmitters at certain sites in the brain, while mania results from a functional excess.

The monoamine hypothesis grew originally out of associations between the clinical effects of various drugs that cause or alleviate symptoms of depression and their known neurochemical effects on monoaminergic transmission in the brain. Initially, the hypothesis was formulated in terms of noradrenaline (norepinephrine), but subsequent work showed that most of the observations were equally consistent with 5-hydroxytryptamine (5-HT) being the key mediator. This pharmacological evidence, which is summarized below, gives general support to the monoamine hypothesis, although there are several anomalies. Attempts to obtain more direct evidence, by studying monoamine metabolism in depressed patients or by measuring changes in
the number of monoamine receptors in post-mortem brain tissue, have tended to give inconsistent and equivocal results, and the interpretation of these studies is often problematic, because the changes described are not specific to depression.

Similarly, investigation by functional tests of the activity of known monoaminergic pathways (e.g. those controlling pituitary hormone releases) in depressed patients have also given equivocal results.

**Table 10: Pharmacological evidence supporting the monoamine hypothesis of depression**

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Principal action</th>
<th>Effect in depressed patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricyclic antidepressants</td>
<td>Block NA and 5-HT reuptake</td>
<td>Mood ↑</td>
</tr>
<tr>
<td>Monoamine oxidase (MAO) inhibitors</td>
<td>Increase stores of NA and 5-HT</td>
<td>Mood ↑</td>
</tr>
<tr>
<td>Reserpine</td>
<td>Inhibits NA and 5-HT storage</td>
<td>Mood ↓</td>
</tr>
<tr>
<td>α-Methyltyrosine</td>
<td>Inhibits NA synthesis</td>
<td>Mood ↓ (calming of manic patients)</td>
</tr>
<tr>
<td>Methyldopa</td>
<td>Inhibits NA synthesis</td>
<td>Mood ↓</td>
</tr>
<tr>
<td>Electroconvulsive therapy</td>
<td>Increases central nervous system responses to NA and 5-HT</td>
<td>Mood ↑</td>
</tr>
<tr>
<td>Tryptophan(5-hydroxytryptophan)</td>
<td>Increases 5-HT synthesis</td>
<td>Mood ↑ in some studies</td>
</tr>
</tbody>
</table>
Treatment of major depression (Craig CR, 1990):

The most common mood disorders are major depression (unipolar depression) and manic-depressive illness (bipolar disorder). Major depression is a common disorder that continues to result in considerable morbidity and mortality despite major advances in treatment. Approximately 1 in 10 Americans will be depressed during their lifetime. Of the 40,000 suicides occurring in the United States each year, 70% can be accounted for by depression. Antidepressants are now the mainstay of treatment for this potentially lethal disorder, with patients showing some response to treatment 65 to 80% of the time.

The mood elevating agents do not act as stimulants of the central nervous system (CNS) with the exception of varying of sedation; the antidepressants have little effect behaviour early in treatment. During this period patients will, however, have side effects specific to the class and agent being used. Only after 2 to 3 weeks of dosing will a therapeutic benefit on depression emerge. At this point the patient begins to demonstrate elevation in mood and self-esteem. In addition, many of the vegetative signs of the illness (e.g., insomnia, anorexia) abate, and the patient regains an interest in daily activities. Failure to continue the medication, however, will result in an immediate relapse into the depressive state. Therefore, maintenance therapy must be continued for at least 6 months.

2.4.3. Antidepressant drugs (Goyal RK, 2007):

Antidepressants are the agents that elevate the mood of depressed individual. The subjects feel more energetic, less sleepy and fresher.

2.4.3.1. Types of antidepressant drugs (Rang HP, 2003):

Antidepressant drugs fall into the following categories –

- Inhibitors of monoamine uptake:
  
a) Non-selective (noradrenaline/serotonin) uptake inhibitors: These include tricyclic antidepressants (TCAs) (e.g. imipramine, amitriptyline) and more recent antidepressants such as venlafaxine (somewhat selective for serotonin,
although less so than selective serotonin uptake inhibitors) and duloxetine, which have fewer side effects than TCAs.
b) Selective serotonin reuptake inhibitors (SSRIs) (e.g. fluoxetine, fluvoxamine and sertraline).
c) Selective noradrenaline uptake inhibitors (e.g. maprotiline, reboxetine).

- Monoamine oxidase (MAO) inhibitors (MAOIs):
  a) Irreversible, non-competitive inhibitors (e.g. phenelzine, tranylcypromine,) which are non-selective with respect to the MAO-A and -B subtypes
  b) Reversible, MAO-A-selective inhibitors (e.g. moclobemide).

- Miscellaneous (atypical) receptor-blocking compounds:
  Its antidepressant actions are poorly understood (e.g. mianserin, trazodone, mirtazapine). The herbal preparation St John's wort, whose main active ingredient is hyperforin, has similar clinical efficacy to most of the prescribed antidepressants. It is a weak uptake inhibitor but also has other actions.

2.4.3.2. Classification of antidepressant drugs (Howland RD, 2006; Lullmann H, 2000)

A) Tricyclic antidepressants (TCA):

- Imipramine
- Trimipramine
- Amitriptyline
- Amoxapine
- Desipramine
- Doxepin
- Meprotilene
- Nortriptyline
- Protriptyline
B) Serotonin reuptake inhibitors:

- Fluoxetine
- Trazodone

C) Monoaminooxidase inhibitors:

- Isocarboxazid
- Phenelzine
- Tranylcypromine

D) Drugs to treat mania:

- Lithium salts

2.4.4. Sites of action of antidepressant drugs (Katzung BG, 2004):

Following schematic diagram is showing some of the potential sites of action of antidepressant drugs (Fig. 11). Chronic therapy with these drugs has been proved to reduce reuptake of norepinephrine or serotonin (or both), reduce the number of postsynaptic receptors, and reduce the generation of cAMP. The MAO inhibitors act on MAO in the nerve terminals and cause the same effects on receptors and cAMP generation.
2.4.5. Adverse effects of antidepressant drugs (Katzung BG, 2004):

Adverse effects of various antidepressants are summarized in following table. Most common unwanted effects are minor, but they may seriously affect patient compliance; the more seriously depressed patient is, the more likely it is that unwanted effects will be tolerated. Most normal persons find that even moderate doses of many antidepressants cause disagreeable symptoms, especially the classic tertiary amine tricyclics: amitriptyline, imipramine, clomipramine, and doxepin. With the SSRIs, transient nausea is the most frequent complaint, and decreased libido and sexual dysfunction create the greatest concerns during maintenance treatment.
### Table 11: Adverse effects of antidepressant drugs

<table>
<thead>
<tr>
<th>S. No</th>
<th>Drug</th>
<th>Adverse effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tricyclics</td>
<td>• Sedation (sleepiness, additive effects with other sedative drugs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sympathomimetic (tremor, insomnia)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Antimuscarinic (blurred vision, constipation, urinary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• hesitancy, confusion)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cardiovascular (orthostatic hypotension, conduction defects, arrhythmias)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Psychiatric (aggravation of psychosis, withdrawal syndrome)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Neurologic (seizures),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Metabolic endocrine (weight gain, sexual disturbances)</td>
</tr>
<tr>
<td>2</td>
<td>Monoamine oxidase inhibitors</td>
<td>Sleep disturbances, weight gain, postural hypotension, sexual disturbances</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(phenelzine)</td>
</tr>
<tr>
<td>3</td>
<td>Amoxapine</td>
<td>Similar to the tricyclics with the addition of some effects associated with the antipsychotic</td>
</tr>
<tr>
<td>4</td>
<td>Maprotiline</td>
<td>Similar to tricyclics; seizures are dose-related</td>
</tr>
<tr>
<td>5</td>
<td>Mirtazapine</td>
<td>Somnolence, increased appetite, weight gain, dizziness</td>
</tr>
<tr>
<td>6</td>
<td>Trazodone, nefazadone</td>
<td>Drowsiness, dizziness, insomnia, nausea, agitation</td>
</tr>
<tr>
<td>7</td>
<td>Venlafaxine</td>
<td>Nausea, somnolence, sweating, dizziness, sexual disturbances, hypertension, anxiety</td>
</tr>
<tr>
<td>8</td>
<td>Serotonin reuptake inhibitors</td>
<td>Gastrointestinal symptoms, decreased libido, sexual dysfunction, anxiety (acutely), insomnia, tremor</td>
</tr>
</tbody>
</table>


Review traces the history of developing models to currently employed genetic models of depression. Over the course of the last 50 years many models of major depressive disorder have been developed on the basis of theoretical aspects of this disorder. These models and procedures have been crucial in the discovery and development of clinically-effective drugs. Present model development is focusing on endophenotypic aspects of behaviours rather than trying to model whole syndromes.
Animals are used in CNS drug discovery in an attempt to reproduce aspects of the behavioural disorders that can be studied, to use as a test bed for discovering novel pharmaceuticals that may treat the disorder and as producers through which new molecular targets can be identified for subsequent drug discovery and development.

Early attempts to model major depression sought to simulate the phenomenology of DSM-IV TR. One of the fundamental problems with modeling behavioural disorders has been the attempts to simulate changes in behaviour without knowing the cause or causes of such abnormalities. Furthermore, it would be impossible to try and simulate all the changes in behavior of animal. Indeed, the initial and traditional pharmacological models of “depression” used in drug discovery were based loosely on behaviours resembling human depression but mostly on changes in behavior induced by specific neurochemical manipulations.

It is interestingly to note that clinically effective medicines for behavioural disorders were discovered initially through serendipity. In the case of depression, the standard tricyclic antidepressant (TCA) imipramine, which is a reuptake inhibitor of monoamines such as nor-adrenaline and serotonin, was initially proposed as a hypnotic tranquilizer to calm schizophrenic patients. Similarly, the prototypical monoamine oxidase inhibitor (MAOI) iproniazid or diaminooxidase inhibitor isoniazids were initially antitubercular drugs.

Reserpine, which depletes these monoamines as well as dopamine, was reported to induce depression in humans who were taking this drug for the treatment of hypertension. These results, among others, helped to formulate the biogenic theory of depression which postulates that depression is associated with central monoaminergic dysfunction.

1). **Neurochemical models of depression** (Arthur R, 2006):

The 1960s was period during which neurochemical models of depression were proposed and refined. The antagonism of pharmacological effects of reserpine was the first model of abnormal monoaminergic function in depression where antidepressant was differentiated from other psychoactive drugs. In addition to reserpine, other
agents with different relative monoamine-depleting specificity such as tetrabenazine, parachloramphetamine and 6-hydroxydopamine were also used.

Psychological effects of reserpine such as ptosis, hypomotality, diarrhoea, bradycardia and hypothermia are readily observed of which hypothermia is most readily antagonized by TCA and MAOI antidepressants.

In keeping with the theory that antidepressants could ameliorate monoaminergic dysfunction, some researchers investigated the interaction between reuptake inhibitors with other indirect or direct monoaminergic receptor agonists. For example, antidepressants were found to potentiate the stimulant effects of amphetamine on motor activity and body temperature. The syndrome of piloerection, hypermotility, irritability, and aggression induced by the catecholamine precursor levadopa is potentiated by antidepressants.

The 1970s saw further refinements in neurochemical models of depression; in particular investigating the relative contribution of one monoamine system over another to depression. For example, it was observed that the dopaminergic receptor agonist apomorphine induces hypothermia stereotype behaviours and vertical climbing in mice. Depending upon the dose of apomorphine, neuroleptics can be differentiated from some antidepressants. Stereotyped behaviours and climbing, but not hypothermia induced by low (1mg/kg) dose of apomorphine are blocked by neuroleptics. On the other hand, the hypothermia induced by high doses (16mg/kg) of apomorphine, but not stereotyped behaviours and climbing are antagonized by tricyclic antidepressants as well as atypical antidepressants nomifensine and viloxazine, but not by trazodone or monoamine oxidase inhibitors.

Likewise, the role of the noradrenergic system in mediating antidepressant activity has been studied using compounds such as yohimbine and clonidine as pharmacological tools. Yohimbine is an alpha-2 adrenoreceptor antagonist that enhances noradrenaline release. In mice this compound increases heart rate and blood pressure in mice leading eventually to death. These effects are potentiated by a wide range of antidepressant drugs such as the TCAs, MAOIs and serotonin reuptake inhibitors (SSRIs). On the other hand, the hypothermia induced by the noradrenaline alpha-2 receptor agonist clonidine can be antagonized by antidepressants.
Parachloroamphetamine has relative specificity for the serotonergic system. Von Voigtlander et al. (1978) as well as Pawlowski (1988) demonstrated that the serotonin depletion and hypothermia induced by parachloroamphetamine was reversed by antidepressants. Nagayama et al. (1980) proposed a model based upon the antagonism of 5-hydroxytryptophan-induced behavioural depression. However, the clinically active antidepressant fluoxetine was found to potentiate these behaviours rather than ameliorate them.

Neurochemical models were also used to investigate receptors thought to be involved in the etiopathology of depression and is thought to be a state marker of the disorder. Changes in β-adrenergic receptors number and sensitivity, for example, have been associated with depression and suicide. It was found that chronic treatment with TCAs could reduce the number of β-adrenergic receptors. However, treatment with atypical antidepressants such as maprotiline, mianserin, zimelidine, nomifensine and bupropion, failed or gave equivocal results on this parameter. It was clear that such behavioural assays would be very efficient in screening for improved enhancers of monoaminergic function, but was unlikely to discover new chemicals entities with novel mechanisms of action.

Thus, the 1960s-70s was also a period that saw the development non-mechanistic based models of depression. That is, models that was not dependent upon the induction of specific neurochemical alterations reversible by specific pharmacological manipulations. Consequently model development concentrated on the replication of some of the changes behaviour thought to be core to the disorder. Stress and psychological modifications induced by stress are thought to be a significant risk factor for the development of depression. Most of these models focused on the phenomenology of behavioural changes induced by stress due to social or environmental manipulations.

2). Ethological models of depression based on social stress (separation, prolonged isolation, social hierarchy):

The separation of infant monkeys from their mothers was one of the earliest models of the effects of social and environmental stress on depression. Infant monkeys react to this separation initially by protesting and finally with behaviours resembling despair.
Similar effects were observed following maternal separation in other animal species. The maternal separation model has evolved considerably in the hands of investigators such as Meaney and his colleagues. Brief periods of separation model has separation of a pup from its dam can induce endocrine responses related to stress such as increased corticotrophin releasing factor and decreased glucocorticoids in various brain areas. These changes, however, are not limited to depressed-like behaviours, but to other behavioural disturbances related to stress (Howland RD, 2006).

Prolonged social isolation was found to uncover muricidal activity in rats and aggression in mice. While aggression, particularly towards mice, might not be considered a core abnormality in depression, nevertheless, forms irritability and aggression in adolescents and these behaviours can be reduced by antidepressants.

Social isolation also induced hyperactivity in rats, which could be related to the disturbances in activity described in DSM-IV TR. This hyperactivity was found to be reduced by some antidepressants.

Stress can be induced not only by social isolation and separation, but also competition within a social milieu. A model of depression based on social hierarchy and subordination was proposed by Malatynsky and Kostowski. Pair housed rats are forced to eat within a limit time, which means that the dominant of the pair alone managed to eat a sufficient amount of food. Repeated treatment with an antidepressant helps the submissive animal to become more assertive and compete longer for food. Antidepressant helped the submissive rats become more assertive. Subsequently, this model was adapted to mice and primates.

Another animal model of isolation and consequent social interaction is the resident – intruder paradigm, in which a rat is placed in the home cage (intruder) of one that has been isolated (resident). The number and type of social interaction between the resident and intruder rat are scored. Resident rats will typically show increased exploration of the intruder, aggression and flight from the intruder rat.

Aggression is reduced by antidepressants such as TCAs, SSRIs and MAOIs as flights from the intruder rats are increased. On the other hand, chronic treatment with
antidepressant will increase rather than decrease aggressive behaviour, which is sensitive to antidepressants.

The resident-intruder paradigm was used by Strekalova et al. (2004) to define aggressive, submissive and neutral mice on the basis of their social interactions. The submissive and aggressive mice were then subjected to various forms of stressors (exposure to a rat, restraint stress and tail suspension) until a decreased preference for a sucrose solution over water was established in the majority of mice. This reduction in response for palatable solutions is considered a measure of the anhedonia, or reduced capacity to drive pleasure in depressed humans, and is an important behavioural read out on present model of development. At baseline all mice had a similar sucrose preference. Following the 4 week period of stress, all of the submissive mice were anhedonic as opposed to less than 40% of the aggressive ones. Anhedonic-like behaviour was also related to the latency to float and the duration of immobility in the rats.

Another variant of the resident-intruder paradigm has been proposed in which rats are deliberately placed in the home cage of heavier, more aggressive rats. The intruder rat is consequently attacked and defeated. The intruder rat can be returned to the aggressor’s cage for repeated social defeats, or can either be put in close proximity to the aggressor or even in the vacant home cage of the aggressor to reinforce this negative experience. There are a number of individual differences in degree of response to this treatment procedure, however, the cause of which have been identified as fighting back, or resisting defeat, housing conditions after the defeat.

Interestingly, it appears that the effects of social defeat are magnified previously aggressive rats that have subsequently been subjected to social defeat. The social defeat experience produces long-lasting behavioural, neurological and neuroendocrinological changes reminiscent of human major depressive disorder such as dexamethasone suppression, impaired 5-HT1A functionality and anhedonic-like behaviours. The impaired reduced intake of sucrose was reversed by imipramine.

While Berton et al (1999) demonstrated that fluoxetine reversed social defeat induced hypophagia in Lewis rats. This paradigm is being used to examine the role of social stress on physiological indices of stress and subsequent behavioural disorders. These
models with high face and construct validity to exposure putative endophenotypes of behavioural disorders

3). Ethological models of depression, based on environmental stress (learned helplessness, forced swimming test / tail suspension, chronic stress):

The learned helplessness model was proposed and developed during the 1960-70s. A presumed state of depression is induced in animals by exposing them to aversive stimuli like shock under circumstances in which they cannot predict the onset or duration of these stimuli. This procedure results in long-lasting deficits in the motivation and ability to escape in subsequent trials where escape is possible, and show behavioural alterations such as vocalizations and passivity. Pharmacological treatment with antidepressants such as imipramine reduces these behavioural changes. This procedure has been refined by Henn and his colleagues, who have described a genetic component to the procedure in that congenital learned helpless rats respond less for a sucrose solution, i.e., were anhedonic.

The forced swimming test (FST), also called behavioural despair or the Porsolt test was first proposed as a simpler variation of the learned helplessness test, and is probably the most widely used screening test of antidepressant potential of novel compounds. Animals are forced to swim in a confined space. They become immobile following a phase of extensive swimming and climbing. Tricyclic and atypical antidepressants reduce the immobility time when the rat is replaced in the cylinder 24 hours following the initial experience. A single test session without a pre-swim session is usually carried out in mice. The FST test has a variant in the tail suspension test (TST) in which mice are suspended by their tail and both the duration of immobility as well as the force of the movements are measured in this test, contrary to the FST SSRIs are active in this test.

FST was originally validated by using the total immobility time. SSRIs are generally inactive and do not alter total immobility time. Subsequently, Lucki and his colleagues modified the observation parameters to include the frequency of immobility episodes as well as type of activity such as swimming and climbing shown by rat as it tries to escape from the cylinder. This refinement of the FST has helped to differentiate antidepressant drugs that work primarily through a noradrenergic
mechanism of action or through serotonin. This procedure is also sensitive to the effects of acute or chronic administration of antidepressants, as well as detecting potential antidepressant activity of non-monoaminergic compounds.

People are more likely to be subjected to periods of stress that wax and wane during their lifetime. Some people are less resilient to these stresses, and can be vulnerable to mild stress- model of depression of seeking to simulate this environmental conditions was initially proposed and developed by Katz and his colleagues who subjected rats to various stressors, such as electrical shock, immersion in cold water, reversal of light/dark cycle, fast, isolation, tail pinch, being shaken, moved from cage to cage over a period of 3 weeks. Following this induction period the rats were exposed to high intensity light and sound that provoked a reduced hypermotility in the stressed animal when compared to non-stressed animals (Craig CR, 1990).

Furthermore, chronically stressed animals reduced their intake of a palatable saccharine solution suggesting that they were impaired in their capacity to drive pleasure form this solution i.e., anhedonic. Tricyclic and atypical antidepressants electroshock and some MAOIs attenuated stress-induced behavioural changes. Variations to extreme stress procedures have been proposed. For example, rats have been forced to run in a running wheel, exhibit low level of motility, which can be ameliorated by imipramine.

The chronic stress model was further developed by Willner and his colleagues whereby rodents are subjected to similar but milder stressors to those used by Katz.

Initially the behavioural read-out variable indicative of anhedonic in this chronic mild stress (CMS) model was sucrose or saccharine intake. However, this has been considered by some to be too variable and other indices of anhedonia-like behavior have been proposed. For example, existing behavioural differences in a supposedly homogeneous group of animals may contribute to the variability of a subsequent measure. As described above, Strekalova et al. (2004) first identified submissive and aggressive animals using the resident-intruder procedure and then showed how these behavioural characteristics could subsequently change hedonic responses in the CMS model. Subsequently, other dependent measure such as changes in feeding and body
weight, responses rates for access to sweetened solutions, or rates of electrical self stimulation have been proposed.

4). Olfactory bulbectomy:

The ethological models and conditions used to induce altered behaviours and the behaviours themselves described above have an intuitive similarity with conditions that are associated with human depressed behaviours, i.e., high face validity. The olfactory bulbectomy model of depression, on the other hand, has little apparent validity. It is difficult to understand exactly how lesions of the olfactory bulbs of rats could be related to depression until one considers the importance detection in the physiological milieu of the rodent, and the subsequent consequences of olfactory bulb ablation on the rodent limbic system and function of the amygdale (Goyal RK, 2007).

For example, bulbectomy results in dysregulation of the limbic hypothalamic axis, increased sensitivity to stress, alterations in immune function, abnormal sleep patterns, agitation, weight loss and changes in hedonic behaviour; changes - that are also seen in depressed patients. The most salient behavioural change following olfactory bulbectomy is increased exploration and hyperactivity in an open field arena that can be attenuated after repeated administration with antidepressants.

5). Operant response models (Vogel HG, 2002):

One of the core symptoms of major depressive episodes is the lack of pleasure which suggests an altered reward system. Consequently, a number of investigators exploited operant response procedures that are maintained by reinforcement as test beds for evaluating antidepressant potential novel compounds. An animal can be trained to respond to a lever for a reward such as water, food and sweetened solutions.

The simplest “schedule of reinforcement” is one where the animal is required to press the lever once for a reward. Other, more complex schedules are then imposed such as progressive ratio where after being trained on a certain number of single responses, the animal is then required to respond, twice, four times, etc. for the same reward. Progressive ratio schedules are used frequently in motivational studies.
Suicide is a common feature of major depression and it can be considered an impulsive act. The differential low rate reinforcement (DRL) schedule of operant responding is a procedure by which impulsivity and inhibitory control can be tested, and is probably the best known operant procedure used to assess antidepressant potential of novel drugs. In this procedure, rats are required to withhold a lever response until a pre-selected time interval since the last reinforcement has elapsed. This interval is usually around 72 s (DRL-72). If the rat responds before this interval has elapsed, the timer is re-set and no reward is given. The schedule of reinforcement has been found to be sensitive to a wide range of antidepressant drugs including the TCAs, MAOIs, SSRIs and the more recently developed dopaminergic and noradrenergic reuptake inhibitors. Rats become more efficient at DRL responding following administration of these drugs by reducing their response rates and subsequently increasing the rate at which they are reinforced.

6). Natural genetic models of depression:

Major depressive episodes and other depressions have a strong genetic basis. Certain rodent strains have been found to exhibit depression like characteristics. Probably the best known natural genetic model of depression is the Flinders rat strain developed by Overstreet and his colleagues.

This strain shows abnormal responses to stress, changes in REM sleep and circadian rhythms and changes in locomotor activity; particularly in the forced swim test. These abnormalities are responsive to TCAs as well as SSRIs. This strain is also reported to show decreased operant responding for water when it has to respond on a progressive ratio schedule.

The Wister Kyoto rats have also been suggested to have behavioural and endocrinological similarities to depressed humans and be responsive to antidepressant drugs.

7). Other tests and models proposed:
Following models have been proposed, but either these have not been further developed, or have been developed for other indications. For the sake of completeness we list these briefly below.

i. Electrical kindling:

There is long history relating convulsions with depression. Electroconvulsive shock was one of the first and most widely used treatments for depression before the discovery of imipramine or iproniazid, and is still in use today for intractable depression. Electroshock can also reverse the behavioural effects in animal models such as learned helplessness, FST, TST, or the resident-intruder paradigm. Kindling is a model of seizures wherein daily low-intensity electrical brain stimulation will sensitize certain brain areas such as the amygdale to the point where seizures will be spontaneously elicited (Vogel HG, 2002).

ii. Chemical convulsants:

Tricyclic antidepressants such as imipramine and amitriptyline have equivocal effects on electroshock or chemically induced seizures. Some investigators such as Fink and Swinyard (1960) suggested that an anticonvulsant effect was characteristic of the chemical class. On the other hand, Barron et al (1965) reported that imipramine potentiated rather than inhibited picrotoxin-induced convulsions was confirmed by Cowan and Harry. Furthermore, similar potentiating effects were observed with nialamide tranylcypromine (MAOI), desmethylimipramine and viloxazine (noradrenergic uptake inhibitors). These results promoted them to propose this test as a functional screen for antidepressants. Roszkowski et al (1976) however, indicated that the effects of tricyclic antidepressants, at least, have a complex effect upon the type of convulsants and seizure. Thus, both imipramine and amitriptyline antagonize maximal tonic convulsions induced by electroshock or pentylenetetrazole with a potency equivalent to standard anticonvulsants such as Phenobarbital, diazepam and hydantoin.

iii. Circadian rhythms and phase-shift:
Altered sleep patterns and circadian rhythms play a major role in mood disorders and on antidepressant drugs. For example, antidepressants increase the readjustment of motor activity after reversal of the light/dark cycle. A related model was later advanced, based on REM sleep in animals. However, no relation was apparent between the ability of antidepressants to suppress REM sleep and their clinical potency. Solberg and colleagues have combined the effects of chronic mild stress (CMS) and exercise as a model of studying altered circadian rhythms and depression. Mice that are subjected to stress and allowed access to a running wheel show depressed-like behaviours including anhedonia and increased immobility in the FST. Exercise through the availability of a running wheel ameliorated the effects of CMS.

8). Present directions on genetic approaches to modeling depressed like behavioural endophenotypes (Howland RD, 2006):

Upto the past decade, animal models concentrated on the induction of depressed-like behaviours such as helplessness, despair or anhedonia through procedure that are intuitively felt to induce depression in humans. On the whole, these models have had high face validity, and are felt to be “reasonable”. Furthermore, this model have been shown to reproduce molecular, biochemical and physiochemical changes seen in depressed humans, thus endowing them with good construct validity.

The cause of behavioural disorders like depression is still very poorly understood and the etiological validity of the model is hypothetical. One of the reason for our limited understanding of the cause of the depression is that it is a complex disorder, which is difficult, if not impossible to model in its entirely, and consequently endophenotypic approaches are being pursued. And endophenotype is defined as heritable characteristic of the illness that is present in affected individual and family members regardless of whether the disorder is active or not. Anhedonia is considered a candidate behavioural endophenotype of depression, as well as other psychiatric disorders such as schizophrenia.

The procedures developed previously to model depressed-like behaviours in animals have subsequently become very important tools in identifying and defining behavioural endophenotypes. FST and TST are not models of the complex behavioural disorder of depression per se, but rather procedures that are capable of
producing changes in behaviour that may be exacerbated by conditions associated with depression, and are sensitive to treatments effective in ameliorating depression in the clinic. This behaviour of immobility bears some resemblance to, but is not identical to fatigue and loss of energy described in DSM-IV TR. These procedures in particular have been used to induce depressed-like behaviours in rodents for subsequent genetic analysis leading to new molecular targets. Similarly, the ability of learned helplessness to characterize specific behavioural abnormalities such as anhedonia, reduced activity, passivity and impaired cognition, which are other putative endophenotypes of depression, suggests its use as a procedure useful in the identification of candidate genes of this disorder.

Other procedures such as olfactory bulbectomy and CMS and the natural genetic models described above are also being used to define behavioural endophenotypes. For example, both the Flinders and Wistar Kyoto rats have been shown to have altered sleep patterns. The Wistar Kyoto rat was also impaired responses to alteration in light-dark cycle. On the other hand, olfactory bulbectomy also alters sleep patterns. These Changes indicate that these rats and procedure may be used to model putative endophenotype of disturbed sleep in depression and other behavioural disorders.

The advent of genetically modified mice in the decade has radically altered the use of animal models of behavioural disorders. Random mutations are being induced by chemical mutagens such as N-ethyl-N-nitrosourea in rodents to alter the genetic makeup. The resulting genetic phenotypes are evaluated through behavioural testing batteries while specific behavioural phenotypes are subsequently confirmed using the very procedures developed as animal models of behavioural disorders including depression. On the other hand, reverse genetic techniques such as the use of transgenic mice are used specially to evaluate the role of candidate genes on the behavioural changes induced or measured by animal models.

In the past decade there has been seen an explosion in the use of genetically modified mice, not only to understand the molecular target and understand the molecular basis of behavioural disorders such as depression, but also in validating molecular drug targets.
Genetic rodent models of depression (Vogel HG, 2002):

Genetic studies have indicated that an individual’s risk of developing depression is influenced by their genetic make-up. Animal models of depression can help the geneticist searching for candidate genes for human genetic studies and also useful in screening antidepressant drugs.

1. Flinders sensitive line rats:

Overstreet et al developed Flinders sensitive Line (FSL) rats at Flinders University in Australia by selective breeding of outbred Sprague Dawley rats for differences in the effects of cholinesterase inhibitor diisopropylfluorophosphate upon core body temperature, water intake and body weight. FSL rats are more sensitive to diisopropylfluorophosphate and cholinergic agonists than are flinders Resistant Line (FRL) rats. FSL rats display exaggerated immobility in the FST, which is reduced by chronic treatment with antidepressants. When subjected to chronic mild stress.

FSL rats display enhanced vulnerability to stress – induced anhedonia. FSL rats also mimic depressed humans in showing altered sleep patterns in the basal state. Brain regional 5-Hydroxy tryptamine (5-HT) levels synthesis, which is altered in depression, has been examined in FSL, FRL and control Sprague-Dawley rats. FSL rats had significantly lower levels of 5-HT levels than the other two. Such reduced state contributes to their depressive features.

2. High DPAT sensitivity rats:

5-HT1A has been implicated in the etiology of anxiety and depression. 5-HT1A receptor antagonist (Buspirone) an anxiolytic and antidepressant drug. Suicide victims showed increased levels of 5-HT 1A receptors. One of its core functional responses is regulation of core body temperature, such that systemic administration of 8-OH DPAT reduces core body temperature. Overstreet et.al created two lines of rats with differential sensitivity to 8-OH DPAT- induced hypothermia.

High DPAT (HDS) sensitive rats are more sensitive to the hypothermic effects of 8-OH-DPAT than are low DPAT sensitive rats. HDS rats also exhibit elevated base line immobility in FST, anxiety like profiles in social interaction tests, and in conflicts.
tests. This supports use of HDS rats as a rodent model of co-morbid depression and anxiety.

3. Congenitally learned helpless rats:

When selectively bred SD rats with high and low propensity to develop earned helplessness H, congenitally learned helpless (cLH) rats showed pronounced LH, whereas as congenitally non-learned helpless (cNLH) animals are highly resistant to LH. cLH have an abnormal stress response, decreased spatial cognitive performance after exposed to stress, altered regulation of serotonin 5-HT-1B autoreceptors and described to show other patterns that might simulate symptoms of depression. Anhedonia was significantly lower in cHL rats and correlated with helpless behaviour.

4. Swim Low-Active rats:

Weiss et.al selectively bred Sprague-Dawley rats to produces rats with either high or low activity in a FST after being exposed to uncontrollable electric tailshock. Swim Low-Active (SwLo) rats show little struggling and much floating in the FST, whereas Swim High-Active (SwHi) rats display the opposite activity. West and Weiss recently described a new procedure that uses the SwLo rat for detecting the effective antidepressants. Chronic treatment with different antidepressants prevented the stress-induced decrease in swim-test struggling shown by both male and female SwLo rats. Chronic treatment with various non antidepressant drugs did not have this effect. Acute (one day) treatment with antidepressants was also ineffective in this test. Therefore, this procedure could be a selective screening technique for effective antidepressant.

5. H/Rouen mice:

Vaugeois, Yacoubi and his colleagues bred CD-1 mice for high and low immobility on the TST, and performed a behavioural, neurochemical and electrophysiological characterization of these lines. After ten generations of breeding, helpless (H/Rouen) mice spent approximately 200s immobile in six minutes TST, whereas, non-helpless
mice (NH/Rouen) spent less than 7s immobile. This difference extended to FST, where H/Rouen mice in generation 14 showed 2-3 times greater immobility than NH/Rouen mice. Such animals tend to exhibit their helplessness/non-helplessness in various other tests (FST, TST, sucrose consumption, sleep wakefulness etc.)

6. High-anxiety-related behaviour mice:

Kromer et al selectively bred CD1 mice for either high-anxiety-relate behaviour (HAB) or low-anxiety related-behaviour (LAB) on an elevated plusmaze test independent of gender, HAB animals were more anxious than the LAB animals in a variety of additional tests, including those reflecting risk assessment behaviours and ultra sound vocalization. Furthermore, in both the FST and TST, LAB animals showed lower scores of immobility than did HAB animals, indicative of a reduced depression-like behaviour. Using proteomic and micro array analyses, glyoxalase-1 was identified as a protein marker that is consistently expressed more highly in LAB mice than HAB animals in several brain areas. Thus, HAB mice represent a promising mouse model of comorbid depression and anxiety.

7. C57BL/6J and C3H/He mice:

Yoshikawa et al. analyzed F2 mice from an intercross between C57BL/6 and C3H/He strains. Composite interval mapping revealed five major loci (suggestive and significant linkage) affecting immobility in the FST and four loci for the TST. The QTLs on chromosomes 8 and 11 overlapped between the two behavioural measures.

In-vitro methods for evaluating mechanism of Antidepressant activity:

Neuronal uptake mechanism for norepinephrine is most important physiological process for removing and inactivating nor epinephrine in synaptic cleft. This uptake is inhibited by cocaine and antidepressants. This mechanism is considered as one of the important modes of action of antidepressant leading to receptor down regulation. In
brain, the hypothalamus shows highest level and greatest uptake of nor epinephrine. Therefore, this region is used for testing potential antidepressant drugs. Similarly sodium dependent transport of 3H dopamine. Dopamine uptake is inhibited by several antidepressants like nomifensine and bupropion, but not by tricyclic antidepressants. Altered serotoninergic function is associated with mood changes in affective disorders. A number of clinically effective antidepressant block the reuptake of 5-HT, in-vitro inhibition of serotonin reuptake in synaptosomes will help to detect compounds that inhibit serotonin uptake into brain synaptosomes likely to be potential antidepressant.

Since the role of adrenoreceptor, (α2) cholinergic receptors, MAO are (likely) to be associated depression/ mechanism of antidepressant effect, several in-vitro models, mentioned below, can be employed to elicit the biochemical mean of antidepressant drugs.

1. Inhibition of [3H]-norepinephrine uptake in rat brain synaptosomes.
2. Inhibition of [3H]-dopamine uptake in rat striatal synaptosomes.
3. Inhibition of [3H]-serotonin uptake in synaptosomes.
4. Binding to monoamine transporters.
5. Antagonism of P-chloramphetamine toxicity by inhibitors of serotonin uptake.
6. Receptor sub-sensitivity after with antidepressants.
8. [3H]-yohimbine binding to alpha-2 adrenoreceptors in rat cerebral cortex.
Cells in the human body use oxygen to breakdown proteins and fats that give them energy. The human body derives its energy from the utilization of nutrients and oxygen as fuel. It also utilizes oxygen to help the immune system, destroys foreign substances and combats diseases.

The byproduct of this and other metabolic process can lead to development of molecular agents that react with body tissues in a process called oxidation. This process is a natural phenomenon of energy generation system and its by-product called free radicals can damage healthy cells of the body.

2.5. ANTIOXIDANTS

An antioxidant can be defined as “any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate” (Halliwell B, 1995). In a biological system they may protect cells from damage caused by unstable molecules known as free radicals.

Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols. They are believed to play a role in preventing the development of such chronic diseases as cancer, heart disease, diabetes, stroke, Alzheimer’s disease, Rheumatoid arthritis, liver diseases and cataracts (Chakraborty P and Hegde KR, 2009).

Antioxidants help in:

- Destroying the free radicals that damage cells.
- Promoting the growth of healthy cells.
- Protecting cells against premature, abnormal ageing.
- Help fight age-related macular degeneration.
- Provide excellent support for the body’s immune system.
Research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity could be measured simply by placing the fat in the closed container with oxygen and measuring the rate of oxygen consumption.

**2.5.1. Classification:** (Chakraborty P, 2009)

**2.5.1.1. Enzymatic antioxidants**

1. Primary antioxidants: e.g.- SOD, Catalase, Glutathione peroxidase.
2. Secondary enzymes: e.g.- Glutathione reductase, Glucose-6-phosphate dehydrogenase.

**2.5.1.2. Non-Enzymatic antioxidants**

1. Minerals: e.g.- Zinc, Selenium
2. Vitamins: e.g.- Vitamin-A, Vitamin-C, Vitamin-E, Vitamin-F
3. Carotenoids: e.g.- Carotene, Lycopene, Lutein, Zeaxanthin
4. Low molecular weight Antioxidants: e.g.- Glutathione, Uric acid
5. Organosulfur compounds: e.g.- Allium, Allyl sulfide, Indoles
6. Antioxidant cofactors: e.g.- Coenzyme Q$_{10}$
7. Polyphenols:
   - Flavonoids –
     - Xanthones – e.g. Mangostin
     - Flavonoids – e.g. Quercein, Kaempferol
     - Flavanols – e.g. Catechin, EGCG
     - Flavanones – e.g. Hesperitin
     - Flavones – e.g. Chrysin
     - Isoflavonoids – e.g. Genistein
     - Anthocyanidins – e.g. Cyanidin, Pelagonidin
   - Phenolic Acid:
     - Hydroxycinnamic acids – e.g. Ferulic, p-coumaric
     - Hydroxybenzoic acid – e.g. Gallic acid, Ellagic acid
   - Gingerol
   - Curcumin
### Table 12: Food sources of antioxidants (Bagchi K, 1998; Beecher G, 2003; Ortega RM, 2006):

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>Fruits (especially citrus) and vegetables, including green and red peppers, tomatoes, potatoes, and green, leafy varieties (e.g., spinach and collard greens).</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Vegetable oils (e.g., soybean, corn, and safflower) and vegetable oil products (e.g., margarine), whole grains, wheat germ, nuts and seeds, and green, leafy vegetables.</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Yellow-orange fruits (e.g., cantaloupe) and vegetables (e.g., carrots) and green, leafy vegetables.</td>
</tr>
<tr>
<td>Polyphenolic antioxidants</td>
<td>Tea, coffee, soy, fruit, olive oil, chocolate, cinnamon, oregano and red wine (Breton F, 2008).</td>
</tr>
</tbody>
</table>

#### 2.5.2. MECHANISM OF ANTIOXIDANTS:

Free radicals are highly reactive molecules or chemical species containing one or more unpaired electrons in their outermost shell. They react quickly with nearest stable molecule to capture the electron, in need to gain stability. They promote beneficial oxidation that produces energy and kill bacterial invaders. If free radicals are at reasonable levels, the human body produces enzymes to combat them and is helpful in immune system and anti-bacterial cell activity (Noguchi N, 2000).

A single free radical can cause damage to millions of other molecules in the body from functioning properly. This molecular destruction is continually occurring in our body. Although antioxidants are a result of breathing but these free radicals attack us from many different sources every day. They are: Alcohol, Tobacco, Drugs, Smoked and Barbecued Foods, Harmful Chemicals and Pesticides, and Food Additives.
2.5.2.1. Antioxidant defense:

Antioxidant defense system (ADS) against oxidative stress is composed of several lines and antioxidants are classified into four categories based on their function (Noguchi N, 2000).

**FIRST:** Preventive antioxidants which suppress formation of free radicals.

**SECOND:** Radical scavenging antioxidants which suppress chain initiation and breaking chain propagation reactions.

**THIRD:** Repair and de novo antioxidants.

**FOURTH:** Adaption where the signal for the production and actions of free radicals induces formation and transport of the appropriate antioxidant to the right site.

2.5.2.2. The antioxidant process (Chakraborty P, 2009):

Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized.

The two ways by which they act are:

- **Chain-breaking** – When a free radical releases or steals an electron, a second radical is formed. This molecule then turns around and does the same thing to a third molecule, continuing to generate more unstable products. The process continues until termination occurs - either the radical is stabilized by a chain-breaking antioxidant such as beta-carotene and vitamins C and E, or it simply decays into a harmless product.

- **Preventive** – Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase prevent oxidation by reducing the rate of chain initiation. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron.
2.5.3. ENZYMATIC ANTIOXIDANTS:

The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), glutathione reductase, thioredoxin reductase, heme oxygenase and biliverdin reductase serve as primary line of defense in destroying free radicals.

Catalase:

An enzyme found in the blood and in most living cells that catalyzes the decomposition of hydrogen peroxide into water and oxygen.

Catalase is a common enzyme found in living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen. Catalase has
one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second.

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme groups which allow the enzyme to react with the hydrogen peroxide. The optimum pH for catalase is approximately neutral (pH 7.0), while the optimum temperature varies by species (Sinha AK, 1972).

Haem-containing catalase breaks down hydrogen peroxide by a two-stage mechanism in which hydrogen peroxide alternately oxidises and reduces the haem iron at the active site.

In the first step, one hydrogen peroxide molecule oxidizes the haem to an oxyferryl species.

In the second step, a second hydrogen peroxide molecule is used as a reductant to regenerate the enzyme, producing water and oxygen.

Some catalase contains NADPH as a cofactor, which functions to prevent the formation of an inactive compound.

Catalases may have another role: the generation of ROS, possibly hydro peroxides, upon UVB irradiation. In this way, UVB light can be detoxified through the generation of hydrogen peroxide, which can then be degraded by the catalase. NADPH may play a role in providing the electrons needed to reduce molecular oxygen in the production of ROS.

Much of the hydrogen peroxide that is produced during oxidative cellular metabolism comes from the breakdown of one of the most damaging ROS, namely the superoxide anion radical (O$_2^-$). Superoxide is broken down by superoxide dismutase into hydrogen peroxide and oxygen. Superoxide is so damaging to cells that mutations in the superoxide dismutase enzyme can lead to ALS, which is characterised by the loss of motoneurons in the spinal cord and brain stem, possibly involving the activation of caspase-12 and the apoptosis cascade via oxidative stress.
The reaction of Catalase in the decomposition of hydrogen peroxide is:

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

**Superoxide dimutase (SOD):**

Superoxide dismutase (SOD) is an enzyme that removes the superoxide (O2−) radical, repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. SOD is found in both the dermis and the epidermis, and is key to the production of healthy fibroblasts (skin-building cells) (Lee SL, 1998).

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

Superoxide Dismutase (SOD) catalyzes the reduction of superoxide anions to hydrogen peroxide. It plays a critical role in the defense of cells against the toxic effects of oxygen radicals. SOD competes with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by scavenging superoxide anions, SOD promotes the activity of NO. SOD has suppressed apoptosis in cultured rat ovarian follicles, neural apoptosis in neural cell lines, and transgenic mice by preventing the conversion of NO to peroxynitrate, an inducer of apoptosis (Beckman JS et al., 1988; Tilly JL, 1995; Keller JN et al., 1998). Covalent conjugation of superoxide dismutase with polyethylene glycol (PEG) has been found to increase the circulatory half-life and provides prolonged protection from partially reduced oxygen species (Beckman JS et al., 1990).

The SOD-catalyzed dismutation of superoxide may be written with the following half-reactions:

\[ \text{M}^{(n+1)+} - \text{SOD} + \text{O}_2 \rightarrow \text{M}^{n+} - \text{SOD} + \text{O}_2 \]

\[ \text{M}^{n+} - \text{SOD} + \text{O}_2 + 2\text{H}^+ \rightarrow \text{M}^{(n+1)+} - \text{SOD} + \text{H}_2\text{O}_2. \]

Where \( M = \text{Cu} \ (n=1) \)

\( \text{Mn} \ (n=2), \text{Fe} \ (n=2), \text{Ni} \ (n=2) \)

In this reaction the oxidation state of the metal cations oscillates between \( n \) and \( n+1 \).

**Superoxide dismutase reaction:**
Superoxide Dismutase

\[ \text{O}_2^- \xrightarrow{\text{Superoxide Dismutase}} \text{O}_2 \]

\[ \text{O}_2 + 2 \text{H}^+ \xrightarrow{\text{Superoxide Dismutase}} \text{H}_2\text{O}_2 \]

Km for O2- for bovine SOD = 0.35 Mm

**Glutathione peroxidases (GSHPx):**

They are a group of selenium dependent enzymes. Four of its isoforms include

1. Cytosolic GSHPx1
2. Plasma GSHPx
3. Phospholipid hydroperoxide PHGSHPx
4. Gastrointestinal GSHPx-GI

All GSHPx require GSH as cofactor and secondary enzymes, such as glutathione reductase and glucose-6 phosphate dehydrogenase for proper functioning. G-6-PDH generates NADPH to recycle the GSH.

\[ 2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O} \]

Mills (1959 and 1957) first demonstrated that this enzyme was present in mammalian red cells and that it protects haemoglobin from oxidative breakdown by hydrogen peroxide. Moderate or severe deficiency of red cells glutathione peroxides is now recognized as a genetically determined enzymopathy (Nechels et al, 1970). The drug induced haemolysis (Boivin et al, 1970; Steinberg et al, 1970 and Stenberg and Necheles et al, 1971) and chronic haemolytic anaemia (Boivin et al, 1969 and Necheles et al, 1970). Hopkins and Tudhope (1973) showed that in patients with carcinoma, there was a wide variation in red cell GSH-Px but the mean value was significantly less than normal’s.
2.5.4. NON-ENZYMATIC ANTIOXIDANTS:

They are classified into two groups:

- Endogenous antioxidants
- Exogenous antioxidants

2.5.4.1. Endogenous antioxidants:

The major extracellular endogenous antioxidants found in human plasma are the transition metal binding proteins i.e. ceruloplasmin, transferrin, hepatoglobin and albumin. They bind with transition metals and control the production of metal catalyzed free radicals. Albumin and ceruloplasmin are the copper ions sequesters. Hepatoglobin binds with hemoglobin, ferritin and transferrin with free iron. Lipoic and uric acids, bilirubin, ubiquinone and glutathione are non-protein endogenous antioxidants which inhibit the oxidation processes by scavenging free radicals. Minerals such as copper, iron, manganese, zinc, selenium enhances the enzymatic antioxidant activities (Vertuani S, 2004; Gale CR, 2001).

Glutathione:

The glutathione (GSH) system is an important endogenous antioxidant system that is found in particularly high concentration in the liver (Kaplowitz, 1985). The importance of GSH for protection against oxidative stress has been shown in the perfused heart (Barracchi, 1984) and in insitu liver (Jaeschke, 1994 and Stein, 1991). Some of the peroxidases are glutathione-dependent and other glutathione-independent. It has been confirmed that the oxidation of GSH by linoleic acid hydroperoxide is enzymatically catalysed in rat liver.

It is suggested that the extremely rapid enzymic reaction of GSH with lipid hydroperoxides break the auto-catalytic chain reactions of lipid peroxidation and thus
protects the vital cellular compounds from the effect of lipid peroxides (Christophersen, 1968). GSH also protects cells from the toxic effect of NO• induced toxicity (Luperchio et al, 1996). Glutathione is present in high concentration as reduced glutathione (GSH) in most of the mammalian cells (Kosower and Kosowerl, 1978). It acts as a nucleophilic scavenger and as substrate in the peroxidase mediated destruction of hydroperoxides. Lipid peroxidation occurs when the cells are severely depleted of GSH (Kyle et al, 1988).

### 2.5.4.2. Exogenous antioxidants:

These are mainly derived from food and other dietary sources. Several herbs, spices, vitamins, foods, vegetables etc exhibits antioxidant activities. Therefore, antioxidants based drugs for the treatment of various pathological diseases have gained attraction in clinical as well as research areas.

Flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, epicatechin, etc found in natural foods are called phytochemicals. Numerous types of bioactive compounds are being used in clinical and preclinical trials from plant sources. Plant derived drugs medicinally useful as it contains terpenoid, alkaloids, glycosides, polyphenolics, steroids which exhibits great importance in research area (Dempster WS, 1995; Wu G, 1999).

### 2.5.5. Role of dietary nutrients in defensive mechanism:

Protein and amino acids are responsible for the synthesis of antioxidant enzymes. GSH and Carnosine are the small peptides, nitrogenous metabolites like creatine and uric acid are the direct scavengers of reactive metabolites (Rassaf T, 2002). Taurine and taurine chloramines effect the iNOS expression and iNOS synthesis in various cells. Dietary deficiency of protein shows hazardous effect in antioxidant system of cell. Arginine and tetrahydrobiopterin deficiency directly affect eNOS which implicates the superoxide production and ultimately, oxidative stress in cells/tissues. Insufficient protein intake effect the availability of Zinc (cofactor of SOD). Plasma
concentration of albumin (zinc transporter) and metallothionein (zinc carrier) is decreased. This results in increase in the iron level in protein malabsorption, as seen in Kwashiorkor (Mohanty P, 2002).

The oxidative stress is due to increase level of tissue iron in protein deficient patient, in which iron-binding protein are deficient including transferrin, lactoferrin and ferritin. This iron overload exhibits the cardiovascular injury. Similarly, high protein diet exhibit oxidative stress. Homocysteine elevation exhibits endothelial superoxide anion in vasculature, increases inducible and constitutive NOS synthesis, and stimulate ROS generation in polymorphonuclear leukocytes and monocytic cells (Garcion E, 1997; Gurujeyalakshmi G, 2000).

**Lipids:**

High intake of polyunsaturated fatty acids is prone to ROS generation, which is neutralized by Vitamin C, E and carotenoids etc. High PUFA intake may increase the risk of cardiovascular diseases. High saturated fat diet increases the risk of iNOS activity in liver and colon. Fish oil contain ω-3 PUFA which is the inhibitor of ROS, iNOS expression and NOS synthesis, decreases the cardiovascular risk by reducing triacylglycerol production in plasma (Sano M, 1999).

**Vitamins:**

Vitamins exhibit anti atherogenic and anti-inflammatory role in the neurons. Vitamin A inhibits iNOS in endothelial cells, vascular muscles cells, cardiac myocytes, mesengial cells. Vitamin D3, K2 and niacin inhibit iNOS activity in brain cells (macrophage, microglia, and astrocytes) (Gurujeyalakshmi G, 2000; Sano M, 1999; Chow CK, 1969). Vitamin E inhibits the ROS generation and thus, prevents the membrane from lipid peroxidation (Yunzhong F, 1983). Irradiation decreases the vitamin C and folate concentration, thus prone to ROS generation. Vitamin B12 and folic acid reduces radical-induced radiation damage and improves leukocytes counts, Vitamin C and Choline prevents DNA damage and hepatocellular carcinoma. One
carbon unit metabolism exhibited by vitamin B12, folic acid and choline, and is an essential participant in methylation of DNA and protein. Vitamin B12, B6 and folate serves as a cofactor for the synthesis of cystathionine synthase and cystothionase (B6), methionine synthase (B12) and also act as a substrate for methionine synthase. These vitamins help to reduce cardiovascular diseases in humans and rats. Similarly, NADP, NADH, FAD, nicotinamide and riboflavin prevent cells from ROS generation. Thiamin is a cofactor of NADPH. NADPH and FAD are required for glutathione reductase, an antioxidant enzyme. NADPH is essential for catalase activity (Yunzhong F, 1984; Hammermueller JD, 1987).

**Micronutrients and Minerals:**

Selenium is a cofactor of glutathione transferase enzyme and other selenoproteins. It has potential antioxidant acitivity and also anticarcinogenic activity, as well. Copper, zinc and manganese are the cofactors of superoxide dismutase enzyme. Deficiency of copper or zinc increases the cytochrome P450 activity in microsomes of liver and lungs, and thus enhances the ROS generation and iNOS expression (Youdim KA, 2000).

**Phytochemicals:**

Phenolic and polyphenolic compounds possess antioxidant activities. These are the natural antioxidants present in grapes, berry crops, tea, herbs, nutmeg, tea, etc. All the herbs and plants contain natural antioxidants compounds including flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechin, isocatechin, gallic acid, esculatin, quercetin etc (Aqil F, 2006; Knuttel H, 2001). Many medicinal plants are considered to have antioxidants activities and contain high content of phenolics like gallic acids and other active constituents. Terminalia chebula, T. bellerica, T. muelleri, and Phyllanthus emblica, Hemidesmus indicus, Cichorium Intybus, Withania somnifera, Ocimum sanctum, Mangifera indica and Punica granatum are known to have potential antioxidant activities (Kirlin WG, 1999).
2.5.6. Effects of dietary antioxidants on clinical outcomes:

Recent studies have suggested that antioxidants may affect clinical outcomes. The Indian Experiment of Infarct Survival Study (Singh RB, 1996) tested the therapeutic efficacy of antioxidants in reducing post-MI complications, many of which are proposed to result from oxidative reperfusion injury. Infarct size and angina and total cardiac events were significantly reduced in individuals receiving antioxidants in the post-MI period. Another potential therapeutic role for antioxidants is in the reduction of restenosis after angioplasty. This role has been addressed in several recent trials (Tardif JC and Yokoi H et al., 1997).

The Multivitamins and Probucol (MVP) Study tested the effects of a combination of vitamin C (1000 mg/d), vitamin E (1400 IU/d), and b carotene (100 mg/d); probucol (a lipid-lowering drug with antioxidant effects; 1000 mg/d); the dietary antioxidants plus probucol (in the same amounts); or placebo alone on the rate and severity of restenosis (Tardif JC, 1997). The Probucol Angioplasty Restenosis Trial (PART) compared probucol (1000 mg/d) with placebo (Yokoi H et al., 1997). In both studies, treatments were initiated 1 month before and maintained for 6 months after elective angioplasty. Probucol significantly reduced restenosis due to its antioxidant properties. In the MVP study, similar results were not observed for the dietary antioxidants, which had no effect alone and appeared to negate the beneficial effects of probucol when given in combination (Tardif JC, 1997). Beneficial effects have been observed for vitamins C and E in other studies (DeMaio SJ et al., 1992; Asad SF, 2001). Because the long-term use of probucol in diseased individuals is of concern, owing to adverse effects on plasma high-density lipoprotein levels (a 41% reduction was noted in the MVP study), dietary antioxidants, could represent a good alternative. Several berries, fruits, nuts, seeds, vegetables, drinks and spices have been found to be high in total antioxidants. The body relies on obtaining its antioxidants from food and other supplements.
2.6. LAGENARIA SICERARIA (LAUKI)

India has a very rich tradition in the use of medicinal plants for the treatment of various ailments. Different medicinal plants come from different important plant families. Out of these important families, one is Cucurbitaceae. The common names for Cucurbitaceae family are the gourd, pumpkin or melon family. Cucurbitaceae consists of one hundred eighteen (118) genera and eight hundred twenty five (825) species. These species are widely distributed in the world. Many of them are economically important domesticated species and many of these have nutritional and therapeutic potential (Rahaman ASH, 2003).

Lagenaria siceraria (Bottle gourd) is an important member of the Cucurbitaceae family. It is a warm-season fruit vegetable. Lagenaria siceraria is cultivated throughout India and Pakistan. The fruits of Lagenaria siceraria are available the whole year in the market. Lagenaria siceraria also known as doodhi, lauki (Hindi), kadoo (Marathi) is official in Ayurvedic Pharmacopoeia. Lagenaria siceraria is an excellent fruit in the nature consisting of all the essential constituents that are necessary for normal and good health of human beings (Rahaman ASH, 2003).

The plant is widely available throughout India. It is a climbing or trailing herb, with bottle- or dumb-bell shaped fruits. Both its aerial parts and fruits are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island etc (Kirtikar KR, 2003). L. siceraria fruit is traditionally used as a cardio protective, cardio tonic, general tonic, aphrodisiac, alternate purgative, anti-inflammatory and diuretic agent (Shivarajan VV, 1996; Kirtikar KR, 2001). It also cures pain, ulcers, fever, and used for pectoral cough, asthma and other bronchial disorders (VV Shivarajan, 1996). Recently, the in vitro antioxidant activity of epicarp and fresh juice of L. siceraria fruit have been reported (Deshpande JR, 2007).
2.6.1. PLANT DESCRIPTION:

Lagenaria siceraria

Scientific Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Cucurbitales
Family: Cucurbitaceae
Genus: Lagenaria
Species: L. siceraria

Synonym: L. leucantha Rusby

Figure 13: Lagenaria siceraria plant with fruits (Deshpande JR, 2007).

2.6.2. Vernacular Names of Lagenaria (Sivannarayana T, 2013):

Hindi: Lauki, ghia
Telugu: Sorakaya, anpakaya
English: Bottle gourd, calabash,
Marathi: Kadoo
Sanskrit: Alaba, tumbi in ancient days
          Iksuaku, katutumbi and mahaphala in recent days
Tamil: Sorakkai
2.6.3 Geographical distribution:

Geographically it occurs throughout India and is now cultivated worldwide. It is generally accepted that Lagenaria siceraria was indigenous to Africa and that it reached temperate and tropical areas in Asia and the Americas about 10,000 years ago, as a wild species whose fruits had floated across the seas or probably with human help (Yash Prashar, 2014).

Derivation of Name:

The word Lagenaria has been derived from the Latin word Lagena, which means Florence flask. While the word siceraria refers to the fruit, which is useful, when it is mature and dry (siccus) (Muhammad Aslam, 2013).

2.6.4 Cultivation and Habitat:

Lagenaria siceraria has both wild and cultivated forms. It is mainly cultivated in countries of African and Asian origin. In India, it is cultivated in various states like Delhi, Uttar Pradesh, Punjab, Haryana, Gujarat, Assam, Meghalaya, Maharashtra, Karnataka and Rajasthan. Lagenaria siceraria is cultivated throughout the year. All types of soil are suitable for cultivation but the best yield is obtained in heavily manured soil. The herb grows best in warm and humid climate. If Lagenaria is cultivated during dry weather then plenty of watering is required. Lagenaria siceraria seeds are sown in two different ways. At first, the seeds can be sown in nursery
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beds, and when there appear two to three leaves on seedlings then they can be transplanted. Secondly, four to five seeds together are sown directly.

The transplantation of seeds is done where there is a desire for early crops. In India, two types of crops are obtained. From mid October to mid March the summer crop is sown whereas the other crop is sown from the beginning of March to the mid of July. In the early crop, round fruits are sown while for latter crop bottle shaped fruits are sown. Then the vines are allowed to trail on the ground, walls, trees or other support. Trailing over give high yields of fruit (Muhammad Aslam, 2013).

**Propagation:** Seeds.

**Useful parts of the plant:** Whole plants are used for medicinal purpose, such as fruits, seed, root, stem and leaves.

### 2.6.5. Pharmacognostical Properties

#### 2.6.5.1. Macroscopy:

Lagenaria siceraria (Bottle gourd) is a pubescent plant. It is a climbing or trailing plant with five angled stems. The tendrils are bifid. Lagenaria has petioled, long leaves with three to five lobes. The fruits can be as long as 1.8 m. The fruits are fleshy and with many seeded pepo. The shape of the fruit is like a bottle. When the fruit is ripe then the epicarp becomes hard like a shell. The fruits contain a large number of smooth seeds, which are white in color. The seeds are 1.6 - 2.0 cm long. As the seeds are horizontally compressed, so they show marginal groove. Lagenaria flowers are solitary, unisexual and chalky white in color. Male flowers have five lobes, five petals and three stamens.

Male flowers are short lived because they remain open only for a few hours, afterward the petals are withered. Female flowers have thick style with three bilobed stigmas (Sivarajan VV, 1996). As far as the opening of the flowers is concerned, both the male and female flowers open at the same time. When we come to the pollination, cross-pollination takes place in bottle gourd because it is a monoecious crop. Honey
bees are the major pollinators (Decker-Walter DS, 2004; Heiser CB, 1979; Sivaraj N, 2005).

2.6.5.2. Microscopy:

Microscopy of transverse section of bottle gourd revealed following characteristics: parenchymatous cells of elongated shape are present in upper epidermis. The cells are covered with cuticle. Parenchymatous cells of elongated shape with wavy walls are present in lower epidermis. A large number of collapsed trichomes are present but glandular trichomes are very few in numbers. The stomata at upper epidermis are also few in numbers. The cells of the mesophyll are circular in shape and they contain chloroplast and arrange themselves in a compact manner forming three to four layers. Vascular bundles are of different sizes and two to three layers of sclerenchyma surround them (Biren NS, 2010; Kirtikar KR, 2001)

2.6.5.3. Phytochemical Properties

**Fruits:** Phytochemicals screening of the edible portion of the fruit of Lagenaria siceraria revealed that it contains 0.2% of protein, 0.1% of fat, 2.9% of carbohydrates, 96.3% of moisture, 0.5% of mineral matter, < 0.01% of phosphorus, and 0.02% of calcium. Reports show that following mineral elements are also present. The values are per 100 g of the herb: iron 0.7 mg, sodium 11.0 mg, potassium 86.0 mg, and iodine 4.5 mcg/kg. Fructose and glucose have also been found. Per gram amino acid composition of the fruit is found to be: phenylalanine 0.9 mg, leucines 0.8 mg, valine 0.3 mg, tyrosine 0.4 mg, alanine 0.5 mg, glutamic acid 0.3 mg, serine 0.6 mg, aspartic acid 1.9 mg, cystine 0.6 mg, cysteine 0.3 mg, arginine 0.4 mg, proline 0.3 mg and threonine 0.2 mg. Vitamin B-complex and Vitamin C (ascorbic acid) have also been detected in the fruit contents. Bitter fruits also contain bitter principles in the form of aglycones.

These principles include cucurbitacins B, D and E (Muhammad Aslam, 2013). The analysis shows that two types of steroids are present in fruit. These steroids are campesterol and fucosterol (Shirwaikar A, 1996). The fresh fruits also contain glucose and fructose in 1:1 ratio. Sucrose was also found in trace amounts. A small amount of
unidentified mono- and dicaffeoylquinic acid derivative was detected (Calabrese N, 2000). As far as the flavonoid complexes are concerned, Lagenaria siceraria contains flavone C-glycosides (Baranowska MK, 1994). In addition, this medicinal plant also contain 3b-O-(E)-feruloyl-D:Cfriedooleana-7,9(11)-dien-29-ol, 3b-O-(E) coumaroyl-D:Cfriedooleana-7,9(11)-dien-29-ol, 3b-O-(E)-coumaroyl-D:Cfriedooleana7,9(11)-dien-29-oic acid, and methyl 2b,3b-dihydroxy-D:C-friedoolean-8-en-29-oate, D:Cfriedooleananetype triterpenes (Chiy-Rong Chen, 2008). The fruiting bodies of Lagenaria siceraria contain a water-soluble polysaccharide, which is composed of 3-O-acetyl methyl-á-d-galacturonate, methyl-á-d-galacturonate, and â-d-galactose in a ratio of 1:1:1. The polysaccharide possesses cytotoxic activity in vitro against human breast adenocarcinoma cell line (MCF-7) (Kaushik G, 2009).

**Seeds:** Reports show that saponins are present in the seeds. The analysis of the seed kernels showed following values: 30.72% of protein, 8.3% of carbohydrates, 2.47% of moisture, 52.54% of oil, 4.43% of ash, 1.58% of fiber, 2.46% of P2O3, and 0.11% of CaO. The color of oil obtained from seed kernels is clear and pale yellow in appearance. Ripe seed kernels gave 45% of the oil. This oil has following features: iodine value 126.6, sap. equivalent, 301.7, 0.55% of free fatty acids and 0.68% of unsaponified matter. Free fatty acid components found to be 18.3% of oleic, 64.1% of linoleic acids and 17.9% of saturated fatty acids (Muhammad Aslam, 2013). Reports show that seeds also contain Lagenin (Wang HX, 2000).

**Leaves:** Leaves contain cucurbitacin B, carbohydrates, phytosterols, saponins, phenolic compounds and tannins, proteins and amino acids and flavonoids (Biren NS, 2010).

**Roots:** The roots contain cucurbitacins B, D, and E and triterpene bryonolic acid (Tabata M, 1993).

**2.6.6. Medicinal uses of different parts:**
Ethnomedicinal uses of various parts of plant:

Traditionally Lagenaria siceraria has been used in the management of various diseases.

**Fruits:** anxiolytic and memory enhancing effect, antihyperglycemic activity, antiulcer activity, adaptogenic property, anti-inflammatory activity, antipyretic, anti-hyperthyroidism, anti-lipid peroxidation, antioxidant activity, antileprosy, cytotoxic, anti-hyper lipidimic activity, immunomodulatory effect and cardioprotective effect.

**Seeds:**Anti-diabetic activity, vermifuge, jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure and skin diseases.

**Leaves:** Anti-asthmatic and anti-allergic activity, hepatoprotective activity, anthelmintic activity, analgesic activity, baldness

**Flowers:** Antidote to poison, ophthalmia and odotalgia

**Stem:** Diuretic

**Roots:** The roots are applied in the treatment of constipation, inflammation and dropsy (Kirtikar KR, 2005)

2.6.7. **Properties and actions mentioned in ayurveda:**

Rasa : Madhura (sweet)

Guna : Snigdha (viscous)

Virya : sita (cool)

Vipaka : Madhura (pleasant)

Karma : Pittahara, Bhedaka, Hrdya, Vrsya

2.6.8. **Ethnoveterinary Usage:**
The fruits, leaves and seeds are used for the treatment of galactischia (Abdullah Al Mamun, 2015), helmintiasis (Awan, M.H, 1981), rabies, trypanosomosis (Firaol Tamiru1, 2013), ruminating and digestive disorders (M. Venkat Ramana, 2008), better lactation (S Bandyopadhyay, 2005), retained placenta, abdominal pain (Tekle Y, 2015) and fever in ruminants.

2.6.9. The reported pharmacological activities of Lagenaria siceraria are as follows:

- The aqueous extract of Lagenaria siceraria has anxiolytic and memory enhancing effect in rodents.
- Aqueous extract of Lagenaria siceraria leaf (LSA) has anti-asthmatic and anti-allergic activity in different animal models.
- Methanol extract of aerial parts of Lagenaria siceraria (MELS) has antihyperglycemic activity in hyperglycemic rats.
- Methanol extract of fruits of Lagenaria siceraria has antiulcer activity.
- Ethanolic extract of leaves of Lagenaria siceraria has hepatoprotective activity against carbon tetrachloride induced liver injury in rats.
- Ethanolic extract of fruit has adaptogenic property on acute stress models of rats.
- A fruit juice of this plant has anti-inflammatory activity against inflammation induced by ethyl phenyl propionate, acitic acid in rats and mice.
- The peel extract of lagenaria siceraria shows regulation of hyperthyroidism, hyperglycemia, and lipid peroxidation in albino mice.
- Different extracts of its leaves has anthelmintic activity against Hymenolepis nana (tapeworm) and pheritima Posthuma (earthworm).
- Different extracts of its fresh and dried fruits have Natural antioxidant activity.
- A fruit of this plant has cytotoxic and free radicals scavenging activity in vitro against human breast adenocarcinoma cell line (MCF-7).
Methanol extract of Lagenaria siceraria fruit has anti-hyper lipidimic activity against experimentally induced hyperlipidemia in rats.

A fruit of this plant has immunomodulatory effect.

The juice of Bottle gourd has traditional property of cardioprotective effect.

Different extracts of its leaves has depressant activity and analgesic activity.

The stem bark and rind of the fruits have diuretic activity.

Different extracts of this seed has diuretic activity in Swiss albino rats.

2.6.10. Other medicinal uses: Other uses include the treatment of cough, asthma, jaundice, kidney stone, colds and measles. Furthermore, the fruits have cooling effect, laxative and diuretic properties (Shah et al., 2010).