1. INTRODUCTION

India is known for its rich heritage of biodiversity and traditional knowledge associated with biological resources. India is one of the top ten mega biodiversity rich nations with four global biodiversity hot spots and shows high endemism. Indigenous resources and traditional knowledge of India has an ancient history, though the codification of traditional medicine varies significantly. Traditional medicinal knowledge in Asia can be divided into, codified systems of ‘traditional medicine’ and the ‘non-codified medicinal knowledge’. The ‘confide traditional medical systems’ of India such as Ayurveda, Siddha, and Amchi are the well written Indian medicinal texts and a part of a time-tested culture, used by people still today. While ‘folk’, ‘tribal’ or ‘indigenous’ medicine refers to non-codified medicinal system, usually transmitted verbally from generation to generation without any written document to a particular geographical region or group of people [1, 2].

History of traditional medicine dates back virtually to the existence of human civilization. Plants since times immemorial have been the basis of traditional medicine systems and key source of medicinal agents. The presently accepted modern medicine (allopathy) has developed gradually over the decades and became the primary choice in developed or urban areas with major scientific and observational achievements of scientists. However, the basis of its progress remains rooted in traditional medicines and therapies [3, 4]. Chemical substances from plants have historically been invaluable as a source of therapeutic agents. Natural products have been used to treat disease since the dawn of medicine. Several drugs have entered in the pharmacopoeias through the study of ethnopharmacology and medicine. According to an assessment nearly 75% of the herbal drugs used worldwide were
integrated from indigenous medicine. In India, approximately 70% of modern medicines are derived from natural resources and numerous other synthetic analogues which have been prepared from prototype compounds isolated from plants [3, 4, 5].

The use of medicinal plants in most developing and poor countries is the normative basis for the maintenance of good health. According to World Health Organisation (WHO), herbal medicine is still the mainstay of about 80% of the people living in the developing countries, for primary health care. In 21st century, herbal medicine has moved from the fringe to the mainstream as greater numbers of people seek relatively safe, cost effective healthcare remedies. The demand of herbal medicines, food supplements, herbal pharmaceuticals, nutraceuticals, and herbal cosmetics are increasing worldwide. Herbal medicine are not only useful in healthcare system but also important economically as the global market for herbal drugs is lucrative, and world herbal trade is expected to reach USD 7 trillion by 2050. Several major pharmaceutical industries have renewed their strategies and interest in favour of drug discovery from natural products, and it is vital to follow the systems of biological applications to facilitate the process [5, 6].

Destruction of forest, industrialization, modern civilization and human interference responsible for rapid declining of medicinal plants, some of them became rare and facing extinction. In recent years attention has been given in relation to the recognition of traditional knowledge as prior art. Combining the strength of the knowledge base of traditional systems with the remarkable power of modern science and technologies will help to find or generate new and effective drug molecules or therapy. Hence plants used in traditional/folk medicinal systems became a primary choice for biological and pharmacological research in modern era.
1.1. TRIPURA AND ITS MEDICINAL PLANTS

Tripura is a small, hilly North Eastern state of India with lovely green hills and valleys, luxuriant forests, streams and small rivers. Tripura possesses an extremely rich bio-diversity and known for its valuable heritage of herbal medicinal knowledge. The state lies between latitudes 22°56’ & 24°32’ N, and longitudes 91°09’ & 92°20’ E. Tripura surrounded by Bangladesh on its north, south and west, and shares national border with Assam and Mizoram on east. Tripura is the third smallest state of the country with a total area of 10,492 km². Geographically state is located in biogeographic zone of 9b-north-east hills, between the river valley of Bangladesh and Mayanmar [7].

Tripura located in a strategic geographical zone with favourable climatic condition (moist to humid), low to moderate temperature (4-38°C) accompanied by good rainfall (2250-2500 mm), which makes Tripura a unique treasure house of medicinal plants. The state is considered as a part of both the Indo-Malayan and Indo-Chinese biological realms, therefore a very close similarity was observed with floral and faunal components of Indo-Chinese and Indo-Malayan subregions. Tripura is a part of both Himalaya as well as Indo-Burma biodiversity which makes the state as a one of the ‘biodiversity hotspots’. According to Forest Survey of India, the state has 7,977 km² of forest area (109 km² very dense forest, 4686 km² moderately dense forest and 3182 km² open forest), which represents 76.04% of state total geographical area. Total geographical area of the state is only 0.32% of India but the state accounts for 12.78% of the total plant species resources found in India [7, 8, 9]. Tripura has been listed as a part of one of the 26 endemic zones in India. The research on status of flora identified 379 species of trees, 320 shrubs, 581 herbs, 165 climbers, 45 epiphytes, 35 ferns, 16 climbing shrubs and 4 parasites (total 1,545 taxa) in the state. Among them nearly 50 plants species restricted to Tripura and its neighbouring
North-Eastern states, out of them 7 species are endemic and 18 are rare species. The State also has 24 species of orchids, of which some are unique in nature. Maximum value of Plant-Diversity Index (Shannon-Weiner) stated in Tripura was 5.23, which is one of the highest index in India [10, 11].

Tripura state ranks second highest in term of population in North-East India. As per Census 2011, the population of the state was 3.67 million (0.30% of the India’s total population) with a density of 350 persons/km². In administrative level, Tripura divided into 8 districts and 23 subdivisions. Tripura has different ethno-linguistic groups, which has given rise to a composite culture. Among total population, Bengali people represents about 69%, while the rest 31% is tribal populations. Tripura is the inhabiting land of 19 different tribes group, which includes Tripuri, Jamatia, Reang, Noatia, Chakma, Bhil, Bhutia, Chaimal, Garo, Halam, Khasia, Kuki, Lepcha, Lushai Mag, Munda, Kaur, Orang, Santhal and Uchai. Each community has their distinct dialect, custom, belief, heritage and socio-religious tradition. A large number of tribes are still living in the hills and dense forests, and depend on Ochai (traditional medical practitioner) or traditional home medicine to meet their health care needs. The use of medicinal plants in the traditional system of folk medicine forms as integral part of the society of the ethnic people of the state [8, 12, 13].

Tripura with rich, old and diverse cultural continuity and ancient traditions have a rich legacy of folk science and traditional knowledge systems. Ethnic people of the state have immense knowledge of their environments and also depend on local flora and fauna for their daily requirements like food, and medicine. These observations not only demonstrated the inventiveness and treasure of food heritage of ethnic people but also their incremental wisdom to sustain the life and ecosystem as a whole. Folk medicinal provided an early benefit to this inhabitant’s science for observing and scrutinizing the knowledge of indigenous people of Tripura is
Figure 1.1. Geographic location of Tripura, India.

[Tripura profile:
- Located between latitudes 22°56’ & 24°32’ N, and longitudes 91°09’ & 92°20’ E
- Surrounded by Bangladesh on its north, south and west, and shares national border with Assam and Mizoram on east
- Total area of 10,492 km².
- 7,977 km² of forest area
- Population - 3.67 million (69% Bengalis, 31% Tribal people)]
based on their necessities, instinct, observation, trial and error and long experience. A rich species diversity of the state has provided an early benefit to this inhabitant's science for observing and scrutinizing the rich natural resources for developing their own traditional knowledge. About 266 species of medicinal plants (68 trees, 39 shrubs, 71 herbs and 88 climbers) have been identified, documented recognised by government of Tripura. Though as per Green Accounting for Indian State Project 2006, state has 1546 flowering plant species and 628 medicinal plants species [10, 14, 15]. The current scenario reveals that Tripura is rich in wild plants with ethnomedicinal value, but a large number of medicinal plant and their folk uses have not documented and remained endemic to certain tribes of the states. This indigenous knowledge is required to be documented for further scientific research and economic development.

Certain specific approach of remedies by using some selected natural plant products are in the field as mentioned below.

1.2. OXIDATIVE STRESS, DISEASES AND ANTIOXIDANTS

1.2.1. Free Radicals and Endogenous Defence

Free radicals are the molecules or molecular fragments containing one or more unpaired electrons in the outer orbit. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the by-products of cellular redox process responsible for playing a both deleterious and beneficial effect in the body. The reactive oxygen and nitrogen radicals includes superoxide (O$_2^-$), hydroxyl (‘OH), peroxyl (ROO’), lipid peroxyl (LOO’), alkoxy (RO’), nitric oxide (NO’) and nitrogen dioxide (NO$_2^+$) radical. Though, hydrogen peroxide (H$_2$O$_2$), ozone (O$_3$), singlet oxygen ($^{1}$O$_2$),
hypochlorous acid (HOCl), nitrous acid (HNO₂), peroxynitrite (ONOO⁻), dinitrogen trioxide (N₂O₃), lipid peroxide (LOOH) are not radicals in nature but can lead to free radical reactions in living organisms. It has been estimated that ~5% of inhaled oxygen is transformed into several harmful ROS species like O₂•⁻, •OH, H₂O₂ by equivalent reduction of oxygen [16, 17].

At low/moderate concentrations ROS/RNS involve in several normal physiological function such as defence against infectious agents by phagocytosis, killing of cancer cells by macrophages and cytotoxic lymphocytes, detoxification of xenobiotics by Cytochrome P450, energy production, cell growth, cell signalling, induction of mitogenic responses, activation of several cytokines and growth factor signalling, release of calcium from intracellular stores, activation of nuclear transcription factors, gene transcription and regulation of soluble guanylate cyclase activity in cells. But overproduction of ROS/RNS involved in the pathological condition. Several enzymatic and non-enzymatic antioxidant defences involved in the regulation of these reactive species produced in animals and humans under physiologic and pathologic conditions [17, 18].

The enzymatic defence system include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and the non enzymatic defence system included vitamin E, vitamin A, vitamin C and reduced glutathione (GSH). SOD is a key endogenous antioxidant enzyme exists in several forms like SOD1 (cytoplasm), SOD2 (mitochondria) and SOD3 (extracellular). It acts as a first line defence system which scavenges superoxide radicals to water and oxygen. CAT is another important antioxidant enzyme can decompose millions of H₂O₂ molecules to water and oxygen in every second.
Figure 1.2. Pathway of generation of reactive oxygen/nitrogen species and site of action of some antioxidants.

[O₂, oxygen; NO, nitric oxide; 'NO, nitric oxide radical; ONOO', peroxynitrite; HOCl, hypochlorous acid; MPO, myeloperoxidase; H₂O₂, hydrogen peroxide; H₂O, water; NADP⁺, nicotinamide adenine dinucleotide phosphate (oxidized); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); L·, lipid radical; LH, lipid (unsaturated fatty acid); LOO·, lipid peroxyl radical; LOOH, lipid hydroperoxide; LOH, form of alcohol; GSH, glutathione; GSSH, oxidised glutathione; SOD, superoxide dismutase; CAT, catalase; O₂⁺, superoxide radical; 'OH, hydroxyl radical.]
Glutathione peroxidase presents in the cytoplasm and eliminate H$_2$O$_2$ by coupling its reduction to H$_2$O with oxidation of GSH. Glutathione reductase a flavoprotein enzyme is essential for the conversion of GSH. Glutathione is a tripeptide which act as a powerful non enzymatic defence system. It is major intracellular nonprotein thiol compound, essential for deoxyribo-nucleic acid (DNA) repair, expression, in maintaining -SH groups in other molecules including proteins, regulating thiol-disulfide status of the cell, and detoxifying foreign compounds and free radicals. GSH is capable to detoxify the hydroxyl radical and singlet oxygen directly, or scavenge hydrogen peroxide and lipid peroxides by the catalytic action of GPx. The major non-enzymatic antioxidants like vitamins C and E can react with free radicals to form less reactive species. They can break free radical chain reactions by trapping peroxyl and other reactive radicals [16, 17, 19, 20].

1.2.2. Oxidative Stress and Human Health

Oxidative stress represents a harmful condition caused by the imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive substance or to repair the resulting damage. Oxidative stress involved in the disturbances of normal redox state of cells and damages of cellular components all components including proteins, lipids, carbohydrates and DNA. Membrane lipid peroxidation, protein oxidation, DNA damage and disturbance in reducing equivalents of the cell are the four critical step of oxidative stress which leads to cell destruction, altered signalling pathways ultimately leads diseases condition. Free radicals induced oxidative stress is now believed to be a fundamental mechanism underlying a number of pathological conditions and aging [17, 21, 22].
A number of mechanisms involved in the pathogenesis of oxidative stress induced diseases such as ‘mitochondrial oxidative stress’ conditions that can illustrate by pro-oxidants shifting the thiol/disulphide redox state and damaging glucose tolerance; and ‘inflammatory oxidative conditions’, an over activity of either NADPH oxidase or xanthine oxidase induced formation of ROS; or by both. Ageing is mainly caused by lipid peroxidation, DNA damage, protein oxidation by free radical action. Every biomolecules of our body are at risk of damage by free radicals and such damaged cell molecules can leads to impair cell functions or cell death resulting in diseased states. The risk of diseases caused by oxidative stress is compounded by unhealthy lifestyle, pollution, cigarette smoking, alcohol, exposure of chemicals, drugs, radiation, stress, illness, and stress etc. Oxidative damage involved in the pathogenesis of a number of diseases such as cardiovascular diseases, disorders in central nervous system (CNS), diabetes mellitus, eye diseases, fertility problem, liver cirrhosis, nephrotoxicity, cancer and aging etc [17, 21, 22].

Antioxidants are the substance that significantly inhibit or delay the oxidative process at low concentration, while often being oxidized themselves. Endogenous and exogenous antioxidants are used to scavenge free radicals and protect the body from reactive species by maintaining radox balance. Human body system is enriched with natural endogenous antioxidant systems, which can avert the onset or advancement of free-radical mediated oxidative stress. Antioxidants through our diet also play an imperative role in helping endogenous antioxidants for scavenging excess free radicals. Hence nutrients, dietary components and food supplements with antioxidant property are vital for the protection against oxidative stress injury [17, 18].
Figure 1.3. Diseases caused by free radicals induced oxidative stress.
Vitamin C, vitamin E, carotenoids, beta carotene, lycopene, N-acetyl cysteine, flavonoids, co-enzyme Q_{10}, alpha-lipoic acid, selenium are the exogenous antioxidant commonly used in dietary and nutritive supplements. Antioxidants like anthocyanins, beta-carotene, catechins, flavonoids, lipoic acid, lycopene, and vitamins C and E are abundant in vegetables, fruits, grain cereals, teas, legumes, and nuts; and intake of a food with antioxidant activity remains the best choice in garnering the benefits of antioxidants [17, 18].

Several researches confirmed that intake of antioxidants in low quantity or low blood levels of antioxidants increases the risk of chronic and acute diseases infect low dietary intake of fruits and vegetables doubles the risk of cancer. Therefore, wholesome antioxidant diet or antioxidant supplements as part of a healthy lifestyle are now being acknowledged to defend the health from oxidative stress. Recently, interest has amplified considerably in finding natural antioxidants for use in foods, cosmetics, or pharmaceutical materials to replace synthetic antioxidants. Synthetic drugs such as butylated hydroxytoluene (BHT), butylated hydroxyl anisole (BHA) etc. are have been reported to cause biological adverse effects such as toxicity, cell damage, inflammations, atherosclerosis and carcinogenic effect in animals and humans. Though some research reports, at normal level synthetic antioxidants seem to pose minimal or no side effect, but tong term use can led to chronic health problems. Traditional medicinal plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. The bioactive antioxidative phytochemicals like alkaloids, phenolic compound, flavonoids, and lignin have received increasing attention for their potential role in prevention of human diseases by suppression or neutralization of free radicals. Phytochemicals from traditional medicinal plants are the major source of antioxidants. The most of these
Phytochemicals are redox active molecules and are the secondary constituents or metabolites found commonly in plants [17, 20, 23, 24]. Different antioxidants and their effect against free radicals are tabulated in Table 1.1.

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Location/Sources</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>Cytosol, mitochondria, nucleus, plasma</td>
<td>Scavenges $O_2^\cdot$ to $H_2O_2$ and water.</td>
</tr>
<tr>
<td>Catalase</td>
<td>Peroxisomes</td>
<td>Scavenges $H_2O_2$ to oxygen and water.</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>Cytosol, mitochondria</td>
<td>Remove $H_2O_2$, lipid peroxides, hydroperoxides, lipoxygenase products by coupling its reduction to $H_2O$ with oxidation of GSH,</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>Cytosol, mitochondria</td>
<td>Reduction of GSSG to GSH in the presence of NADPH. Reduction of low molecular weight disulfides.</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Cytosol</td>
<td>Maintaining -SH groups and other molecules including proteins, regulating thiol-disulfide status of the cell, and detoxify $O_2^\cdot$, 'OH, RO' and reduce $H_2O_2$ directly to water.</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Wide distribution</td>
<td>Scavenge singlet oxygen, $O_2^\cdot$, 'OH, RO', and binds transition metals.</td>
</tr>
<tr>
<td>CoQ$_{10}$</td>
<td>Human cells, organ meats, fish, wheat bran</td>
<td>It reduce lipid peroxidation, reduces mitochondrial oxidative stress, and also able to recycle vitamin E.</td>
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<td></td>
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<tr>
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<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Cysteine</strong></td>
<td>Wide distribution</td>
<td>Vital component for the synthesis of glutathione. Scavenges of H$_2$O$_2$ and peroxide.</td>
</tr>
<tr>
<td><strong>Transferrin</strong></td>
<td>Wide distribution</td>
<td>Bind with free iron salts, thus prevent the generation of ROS.</td>
</tr>
<tr>
<td><strong>Lactoferrin</strong></td>
<td>A milk protein found extracellularly</td>
<td>Bind with free iron salts, thus prevent the generation of ROS.</td>
</tr>
<tr>
<td><strong>Ceruloplasmin</strong></td>
<td>Extracellularly</td>
<td>A copper binding protein, and catalyses the oxidation of Fe$^{2+}$ to Fe$^{3+}$</td>
</tr>
<tr>
<td><strong>Bilirubin</strong></td>
<td>Blood, plasma and extravascular place.</td>
<td>Scavenges peroxyl radicals and defend albumin-bound linoleic acid from oxidation caused by peroxyl radical.</td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
<td>Cells, mitochondrial membranes. Citrus fruits, nuts, olive &amp; groundnut oil.</td>
<td>Scavenges of superoxide, hydroxyl radicals directly, and upregulation of antioxidant enzymes, breaks lipid peroxidation chain reactions.</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
<td>Intracellular and extracellular fluid, lemon, oranges, palm and olive oil, germinated pulses</td>
<td>Scavenges superoxide radicals, hydroxyl radicals, and neutralize oxidants from stimulated neutrophils, regenerates vitamin E</td>
</tr>
<tr>
<td><strong>Phytochemicals</strong> (Phenolic compounds, Flavonoids, Alkaloids, Triterpenoids)</td>
<td>Vegetables and different plants</td>
<td>Scavenges free radicals directly or inhibit the generation of free radicals by specific mechanism depends upon the nature of phytochemicals</td>
</tr>
</tbody>
</table>
1.3. DIABETES MELLITUS

Diabetes mellitus (DM) represents a group of complex heterogeneous disorder characterized by hyperglycemia and glucose intolerance, due to insulin deficiency, impaired effectiveness of insulin action or both. It is one of the most common chronic endocrine disorders that results significant impact on health, excellence and expectancy of human life as well as on health care system. Diabetes mellitus is classified on the basis of aetiology and clinical presentation of the disorder into four types [25, 26, 27, 28],

![Types of Diabetes](image)

**Figure 1.4. Types of diabetes mellitus**
DM became fourth or fifth leading cause of death in most developed countries, and became pandemic in many developing and newly industrialized nations. Diabetes patients are at a risk of development of acute metabolic, eye, foot, cardiovascular, digestive, skin, kidney, oral, dental, sexual, pregnancy related, nerve, infective, CNS related, thyroid, autoimmune complication and disability [25, 26, 27].

1.3.1. Diabetes Epidemiology and India

As per WHO statistics, between 2000 and 2030 world population will increase by 37%, but the number of people with diabetes will increase by 114% [29, 30]. India, China and USA are the top most countries, in numbers of people with diabetes. It was estimated that in the year 2000, about 31.7, 20.8, 17.7 million people was affected by diabetes in India, China and USA respectively, which will cross 79.4, 42.3, 30.3 million mark by 2030 in respective countries [30]. According to International Diabetes Federation (IDF), the prevalence of diabetes and impaired glucose tolerance (IGT) in the age group 20-79 years was 5.9% and 7.5% in 2007, which are envisaged to cross 7.1% and 8.1% by 2025, of which about 80% people lives in developing countries [31]. New statistics showed that, the number of diabetic people in 2011 has reached a stunning 366 million which is set to rise 552 million by 2030 globally, but approximately 183 million people (50%) are still unaware about their diabetic condition [32]. It was estimated that global health expenditures to treat and prevent diabetes and its complications were nearly USD 232.0 billion in 2007, which will exceed USD 465 billion in 2011, which was 11% of total expenditures in adults (20-79 years). Diabetes mellitus is pandemic in both developing and developed countries with increased rate of morbidity and mortality; as in 2011, diabetes caused 4.6 million deaths [32, 33].
Diabetes is probably one of the well described disorders in ancient India as “Madhumeha”. The oldest reference to diabetes in ancient Indian texts dates back to 4500 years. The Charaka Samhita, the ancient Indian medical treatise succinctly explains about the etiopathogenesis, symptomatology, complications and treatment of prameha (metabolic disorders) and madhumeha. It also suggested that being obese was a major risk factor for diabetes [34, 35]. India leads the world in the number of diabetic population, and often referred diabetic capital of the world with 61.3 million diabetic people currently. The percentage prevalence of diabetes and percentage IGT prevalence in India was 6.2 and 5.4 in 2007, which was set to raise 7.6 and 6.1 respectively in 2025 among the people of age between 20-79 years [30, 31, 32].

1.3.2. Insulin and Carbohydrate Metabolism

Carbohydrates from dietary sources serve as the principal exogenous source of glucose. Glucose is considered as main fuel for energy requirement and necessary to ensure proper function and survival of all organs. The impairment is glucose metabolism may lead to physiological imbalance and warrants proper management. Breakdown of carbohydrates by digestive enzymes causes the released glucose, which serve as primary stimulus for the β-cells of the pancreatic islets to release insulin. Insulin is the key hormone controlling intermediary metabolism and blood glucose level. The islets of langerhans of pancreas contain mainly four cell types, β-cells secrete insulin, A- (or α) cells secrete glucagon, D-cells secrete somatostatin and PP-cells secrete pancreatic polypeptide. Insulin is synthesized as a preproinsulin (precursor) in the rough endoplasmic reticulum is transported to the golgi apparatus where preproinsulin undergoes proteolytic breakdown first to proinsulin and then to insulin and C-peptide, which are stored in granules in β cells [36, 37, 38]. Release and action of insulin was given in Figure 1.5.
Figure 1.5. Release of insulin and its action.
Glucose enters β-cells via a membrane transporter called glut-2, and its subsequent metabolism via glucokinase and glycolysis raises intracellular adenosine triphosphate (ATP). This blocks K<sub>ATP</sub>, which results membrane depolarisation and opening of voltage dependent calcium channels, leading to Ca<sup>2+</sup> influx and results insulin secretion. Insulin influences the glucose metabolism in most tissues, in liver it inhibits glycogenolysis and gluconeogenesis and stimulates glycogen synthesis. It glycolysis, but the overall effect is to increase hepatic glycogen stores, and increase fatty acid as well as triglycerides synthesis in adipose tissue and liver. Insulin restrains lipolysis, protein catabolism, and oxidation of amino acids in the liver [36, 37, 38].

1.3.3. Pathogenesis of Diabetes

People with impaired glucose homeostasis are at increased risk of developing diabetes. Plasma glucose level is usually sustained within a narrow range reflecting a delicate balance between glucose production and glucose utilization. Therefore maintenance of ‘glucose homeostasis’ is essential [36, 37, 38, 39].

Type 1 DM (insulin dependent diabetes mellitus, IDDM) is believed to have an autosomal recessive inheritance and autoimmune pathogenesis. Genetic susceptibility, autoimmune factors and environmental factors (like, viral infection, diseases condition, geographic or seasonal variation) are involved in the pathogenesis of IDDM. About 20 different regions of the human genome have been identified to have some degree of linkage with IDDM. Type 2 DM (non insulin dependent diabetes mellitus, NIDDM) is caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors such as obesity, overeating, lack of exercise, and stress, as well as aging. It is typically a multifactorial
disease involving multiple genes and environmental factors to varying extents. The number of diabetic patients is increasing rapidly reflecting the changes in lifestyle [36,37, 38, 39].

Figure 1.5 describes mechanism involved in the pathogenesis of DM.

![Diagram of mechanism involved in the pathogenesis of DM](image)

**Figure 1.6. Mechanism involved in the pathogenesis of IDDM and NIDDM.**
1.3.4. Diagnostic Criteria

The current diagnostic criteria as per WHO for the diagnosis of diabetes and intermediate hyperglycaemia have been used commonly and accepted widely. Plasma glucose level stills the key criteria for diagnosis of diabetes. However, American Diabetes Association (ADA) in 2003 modified its recommendations resulting in few discrepancies between its recommendations and those of the WHO. Table 1.2 is describing the diagnostic criteria for diabetes proposed by WHO and ADA [40, 41].

Table 1.2: Diagnostic criteria for diabetes mellitus

<table>
<thead>
<tr>
<th>WHO recommendation</th>
<th>ADA recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes:</strong></td>
<td>A1C ≥ 6.5%. (The test method should be NGSP certified and standardized to the DCCT assay).# or</td>
</tr>
<tr>
<td>FPG: ≥ 126 mg/dl (7.0 mmol/l) or</td>
<td>FPG$ \geq 126 \text{ mg/dl (7.0 mmol/l)}$.# or</td>
</tr>
<tr>
<td>2–h plasma glucose $^* \geq 200 \text{ mg/dl (11.1 mmol/l)}$</td>
<td></td>
</tr>
<tr>
<td><strong>Impaired Glucose Tolerance (IGT):</strong></td>
<td></td>
</tr>
<tr>
<td>FPG: &lt; 126 mg/dl (7.0 mmol/l) and</td>
<td></td>
</tr>
<tr>
<td>2–h plasma glucose $^* \geq 140 \text{ and } &lt; 11.1 \text{ 200 mg/dl (7.8 and 11.1 mmol/l)}$</td>
<td></td>
</tr>
<tr>
<td><strong>Impaired Fasting Glucose (IFG):</strong></td>
<td></td>
</tr>
<tr>
<td>FPG: 110 to 125 mg/dl (6.1 to 6.9 mmol/l)</td>
<td></td>
</tr>
<tr>
<td>and (if measured)</td>
<td></td>
</tr>
<tr>
<td>2–h plasma glucose $^* \leq 140 \text{ mg/dl (7.8 mmol/l)}$</td>
<td></td>
</tr>
</tbody>
</table>

*$\text{Venous plasma glucose 2–h after ingestion of 75g oral glucose load.}$

If 2–h plasma glucose is not measured, status is uncertain as diabetes or IGT cannot be excluded.

FPG: Fasting plasma glucose; OGTT: Oral glucose tolerance test; NGSP: National Glycohemoglobin Standardization Program.

#In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

$\text{Fasting is defined as no caloric intake for at least 8 h.}$

**Test should be performed as described by the WHO.
1.3.5. Chemical Used in the Induction of Experimental Diabetes

Alloxan and streptozotocin (STZ) are commonly used to induce experimental diabetes in animals. Toxic effect on β cell of both these diabetogenic agents is mediated by ROS. Alloxan was rapidly taken up by the pancreatic β cell followed by the generation of ROS. Alloxan and dialuric acid (a product of alloxan reduction) establish a redox cycle with the formation of superoxide radical [42, 43].

Superoxide radical undergo dismutation to H₂O₂, followed by the formation of hydroxyl radical by the Fenton reaction. Toxic effect of these ROS with a concurrent massive increase in concentration of cytosolic calcium results rapid destruction of β cells which leads to type 1 diabetes. Some researches reported that high fat diet followed by alloxan may cause type 2 diabetes [42, 43].

Streptozotocin [2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose] is used widely to induce both type 1 and type 2 diabetes. STZ penetrates the β cell via GLUT2 and leads to alkylation of DNA. DNA damage cause activation of poly adenosine diphosphate (ADP-ribosylation, a method that is more significant for the diabetogenicity of STZ than DNA damage itself. The poly ADP-ribosylation causes depletion of cellular NAD⁺ and ATP. Increased ATP dephosphorylation after STZ administration provides a substrate for xanthine oxidase which generates superoxide radicals [42, 43].

Consequently, ROS like hydrogen peroxide and hydroxyl radicals are also produced during the process. In addition, STZ liberates toxic amounts of nitric oxide (NO) that restrains aconitase activity and participates in DNA damage, which leads to β cells destruction by necrosis [42, 43].
1.4. LIVER AND HEPATOTOXICITY

Liver is the heaviest and largest internal organ, weighing about 1.4 kg in the adult. Liver is located directly below the diaphragm, and occupies the right hypochondriac, epigastric, umbilical regions and part of the left hypochondriac. Liver consists of two major lobes, right lobe (larger in size) and left lobes (smaller in size), and other two minor lobes are caudate lobe and quadrate lobe [44, 45].

Hepatocytes are the foremost functional cells of the liver, involved in metabolic, secretory, and endocrine functions. These are specialized epithelial cells arranged in single file and form about 80% of the volume of the liver. Centre vain locates at the centre, and joins other central veins to form the hepatic vein. Bile canaliculi are the small ducts between hepatocytes that collect bile produced by the hepatocytes. Bile passes into bile ductules from bile canaliculi, and then in bile ducts [44]. Hepatic sinusoids are highly permeable blood capillaries that accept oxygenated blood from hepatic artery branches and nutrient-rich deoxygenated blood from hepatic portal vein branches. The sinusoids consisting of two cell populations: (a) extremely thin, sparse endothelial cells, involved in immunoreactivity and (b) Kupffer cells, which phagocytose numerous substances, such as latex particles, denatured albumin, bacteria and immune complexes [44, 45, 46].

1.4.1. Physiological Function of Liver

Liver is involved in nearly 500 body functions, and plays a significant role in digestion, metabolism, and immune defence. Different some physiological functions of liver are [44, 45, 46, 47].
- **Metabolism:** Carbohydrates metabolism and maintenance of glucose hemostasis, lipid metabolism, protein metabolism.

- **Synthesis/activation:** Synthesis of glucose, cholesterol, lipoproteins, plasma proteins (alpha and beta globulins, albumin, prothrombin, and fibrinogen.), bile salts, blood proteins (albumins, fibrinogen, globulins, heparin), and angiotensin. Activation of vitamin D.

- **Detoxification:** Detoxification of alcohol, toxins, drugs, and excretes drugs (i.e. penicillin, erythromycin) into bile. Chemically modify or excrete thyroid hormones and steroid hormones such as estrogens and aldosterone.

- **Storage:** Storage of vitamins (A, B₁₂, D, E and K), minerals (iron, copper), glycogen, fats.

- **Secretion:** Secretion of thrombopoietin, hepcidin, and insulin-like growth factor-I.

- **Excretion:** Bilirubin, cholesterol excretion.

- **Immune mechanism:** Removal of pathogens and worn out red blood cells.

### 1.4.2. Liver – Detoxifying Agent

Liver plays a crucial role in detoxifying a variety of harmful substances, including alcohol, drugs, chemicals, pesticides, solvents, and heavy metals. On exposure to high levels of harmful chemicals, the liver can become overwhelmed, and toxins are transported to the liver by the portal vein. Liver detoxifies these toxic substances and excretes them in the bile. Along with detoxification of exogenous compounds, liver also plays a vital role for detoxifying and metabolizing endogenous compounds. The liver excretes toxic by-products of normal metabolism (i.e.
ammonia) and excess hormones (i.e. estrogen). Liver is the most significant place of drug metabolism, although extrahepatic metabolism (in gastrointestinal mucosa and by circulating enzymes such as esterases) of drugs is also well recognised [48, 49, 50].

Enzymes responsible for such action are mainly expressed in hepatocytes and can be divided in two groups Phase I and Phase II. Phase I metabolism causes basic structural alteration of a drug molecule, whereas phase II metabolism causes conjugation of a hydrophilic chemical moiety to drug molecule. P450 enzymes are the principal catalysts of hepatic phase I metabolism. P450s include a gene superfamily with 57 members in the human genome. A subset of approximately fifteen P450 enzymes from CYP1, 2 and 3 gene families arbitrate 70–80% of all Phase I-dependent metabolism of therapeutic drugs and contributes in the metabolism of countless other xenobiotic chemicals. P450 enzymes from other families are responsible for synthesis of cholesterol, steroid synthesis and fatty acid metabolism. Enzymes involved in Phase II metabolism are collectively known as ‘transferases’, as they catalyse the transfer of a moiety from a donor molecule to the drug. Some of these enzymes are glutathione, S-transferases, sulphotransferases, N-acetyltransferases, DP-glucuronosyltransferases [48, 49, 50].

1.4.3. Liver Toxicity

Liver plays a fundamental role in transforming and eliminating endogenous and exogenous agents, but in the same time liver is susceptible to the toxicity from various such agents. Xenobiotics or toxic chemicals directly or indirectly may cause damage or alter the cellular functions of liver. Faster accumulation of toxins in the
liver can lead to liver damage or hepatotoxicity. Certain xenobiotics, alcohol consumption, malnutrition, infection, anaemia and medications are responsible for liver damage. An obvious sign of hepatic injury is the liver cell membrane is damaged causing leakage of variety cellular enzymes that are released into the blood stream from cytosol, thereby causing an increased enzyme level in the serum, so it may not able to perform it functions optimally [51, 52]. Some common causes of hepatotoxicity are [53, 54, 55, 56]:

A. Therapeutic Drugs
Non steroidal anti-inflammatory drugs (NSAIDs) (Diclofenac, Paracetamol, Aspirin), Anti-gout drug (Allopurinol), Antibacterial agents (Flucloxacillin, Erythromycin, Ciprofloxacin, Penicillins, Sulphonamides, Macrolide antibiotics, Tetracycline), Anti-TB drugs (Isoniazid, Rifampicin, Pyrazinamide), antiviral drugs (Ritonavir, Ketoconazole), Immunosuppressants/anticancer agents (Azathioprine, Busulfan, Methotrexate, Melphalan), Anti-arrhythmic drugs (Amiodarone), Antihypertensive drugs (Methyldopa, Losartan), Lipid lowering agents (Ezetimibe, Statins), Antiplatelet agent (Clopidogrel), Anti-epileptics agents (Phenytoin, Carbamazepine, Valproic acid), Psychiatric drugs (Chlorpromazine, Paroxetine), Antidepressant drugs (Fluoxetine, Paroxetine), General anaesthetics (Halothane), Antidiabetic drugs (Sulphonylureas, Acarbose), Steroids (Androgenic steroids, Contraceptive steroids, Anabolic steroids), antiandrogen drug (Flutamide), Atacids (Omeprazole),

B. Non-drug causes of liver impairment
Viral hepatitis (Hepatitis A, B, C, E virus), alcohol, biliary tract diseases, autoimmune diseases (Autoimmune hepatitis and Primary biliary cirrhosis), haemodynamic disorders (Heart failure, Ischaemia/ hypoxia, Budd-Chiari syndrome, Portal vein thrombosis, Veno-occlusive disease), metabolic/ genetic diseases (Wilson's disease, Haemochromatosis, Sepsis-induced cholestasis)
1.4.3.1. Drug induced liver injury

Drug-induced liver injury (DILI) represents liver damage caused by the diverse therapeutic drugs, herbal medicines, plants, or nutritional supplements. DILI is the very common reason for drug withdrawal and may cause acute liver failure, liver transplant, or fatality. Several researches suggest that DILI is responsible for approximately 10% of all acute hepatitis cases, over 50% of acute liver failure, and 4% of hospital admissions for jaundice. A number of drugs like Ticrynafen, Alpidem, Tolrestat, Tolcapone, Troglitazone, Aminoptine, Pemoline, Trovafloxacin, Ximelagatran were withdrawn from market due to hepatotoxicity. Based on the criteria given by Council for International Organizations of Medical Sciences, DILI can be divided into 3 types [48, 55, 57, 58],

1) **Hepatocellular**: Characterized by marked increase in serum aminotransferase (ALT), previous total bilirubin elevation and moderate increase in alkaline phosphatase (ALP). This type of DILI caused due to an adverse reaction primarily affecting hepatocytes as a result of binding of a parent drug or its metabolites to hepatic cellular proteins or macromolecules, and related with the large incidence of acute liver failure, which includes jaundice end up with liver transplant or death. It can be diagnosed as, \( \text{ALT} > 2 \text{ ULN} \) or \( R \geq 5 \),

Where, ULN, upper limits of normal; \( R = \) serum activity of ALT/serum activity of ALT, both of which are expressed as multiples of the ULN.

2) **Cholestatic**: Characterized by predominant increase in ALP. Pathology of cholestatic DILI includes binding of the drug or its metabolites to protein at the canalicular membrane contribute to ductal injury. It can be diagnosed as, \( \text{ALP} > 2 \text{ ULN} \) or \( R \leq 2 \)
Mixed: Characterized by increase in both ALT and ALP. ALT is usually 2 to 5 times higher than ALP. Clinical and biological characters of mixed DILI falls between the 2 other types. It can be diagnosed as, ALT > 2 ULN and 2 < R < 5.

Disruption of the cell membrane and cell death caused by the covalent binding of the drug to cell proteins and to inciting an immunologic reaction, inhibition of cellular pathways of normal drug metabolism, abnormal bile flow caused by the disruption of subcellular actin filaments or interruption of transport pumps, apoptosis, inhibition of mitochondrial function, caused be oxidative stress, lipid peroxidation, fat accumulation are the major factors responsible for hepatotoxicity [59].

Two possible reasons to the pathogeneses of DILI include direct hepatotoxicity (intrinsic hepatotoxicity) and idiosyncratic reactions (idiosyncratic hepatotoxicity). Idiosyncratic hepatotoxicity can be classified further as allergic and non-allergic [55, 57].

- **Intrinsic hepatotoxicity** is predictable, dose-dependent, and host-independent and has an early onset or short latency period. This type of hepatotoxicity can be aggravated by several factors such as age, gender, body mass index, pregnancy, renal or hepatic disease, and concomitant use of any CYP 450 inducers (ie, phenobarbital, ethanol, cigarette smoke, and grape juice).

- **Idiosyncratic hepatotoxicity** is unpredictable, host dependent and occurs without obvious dose-dependency, and has intermediate (1-8 weeks) or late latency (up to 12 months) periods. This type of DILI characterises immune-mediated hypersensitivity, and genetic predisposition in susceptible individuals may one of the most underlying reason in this cases.
Figure 1.7. Mechanism of drug induced liver toxicity
1.4.3.2. Alcohol induced liver damage

Alcohol is the most frequent cause of liver injury in the developed world and the incidence is increasing considerably worldwide. Consumption of alcohol is associated with a variety of diseases in the liver ranging from steatosis, steatohepatitis to cirrhosis and hepatocellular carcinoma. The effects of acute and chronic alcohol ingestion significantly depend on the concentration and dose of the alcoholic beverage along with various other factors, such as nutritional status, gender and ethnicity. Alcoholic liver disease (AID) can be characterized in three overlapping, distinct pathological and clinical features like fatty liver (or steatosis), alcoholic hepatitis and cirrhosis [60, 61].

Though, the pathophysiology of AID is complex and not fully understood, but the pathogenesis of acute and chronic alcohol consumption is multi-factorial with varied consequences in different cell types. Alcohol-induced injury takes place at multiple levels and can effects the innate immune cells to the liver parenchymal cells, hepatocytes. The innate immune cells including Kupffer cells play a crucial role in early liver injury caused by alcohol through recognition of endotoxin/lipopolysaccharide in the portal circulation [60, 61, 62, 63]. The advancement of alcohol-induced liver damage involves parenchymal cells and macrophages through the direct and indirect effects of alcohol and its metabolites, oxidative stress, immunologic and inflammatory events. Metabolism of alcohol to acetaldehyde and then to acetate in the presence of their respective dehydrogenases leads to the formation of nicotinamide adenine dinucleotide (NADH), which restrains fatty acid oxidation and promotes fat accumulation. Alternative metabolism of alcohol
by the cytochrome P450 enzyme leads to the generation of ROS, which is responsible for lipid peroxidation and inflammation [60, 61, 62, 63].

1.4.4. Liver enzyme

The hepatotoxins generate a wide variety of clinical and histopathological indicators of hepatic injury. Liver injury can be identified by certain biochemical markers. Alanine aminotransferases (ALT) or serum glutamic pyruvic transaminase (SGPT) activity is the standard biomarker of hepatotoxicity. ALT plays a significant role in amino acid metabolism and gluconeogenesis. Normal level is 5-50 U/L. Aspartate aminotransferases (AST) or serum glutamic oxaloacetate transaminase (SGOT) is another important liver enzyme that helps in producing proteins. Normal level is 7-40 U/L. Alkaline phosphatase (ALP) is a hydrolase enzyme that is removed through bile. It is responsible for monophosphates hydrolysis at an alkaline pH. Normal level is 20-120 U/L. Bilirubin is an endogenous anion produced from the regular degradation of haemoglobin from the red blood cells (RBC) and excreted in the bile from the liver. Normal bilirubin level is 0.2 to 1.2 mg/dl [64]. Some of the biochemical markers of hepatotoxicity are listed in Table 1.3 [64].

<table>
<thead>
<tr>
<th>Biochemical markers</th>
<th>Causes for abnormalities and histological lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, AST, Lactate dehydrogenase, Sorbitol dehydrogenase, Serum F protein, Arginase I, Glutamate dehydrogenase, Malate dehydrogenase,</td>
<td>Leakage from damaged tissues - Hepatocellular necrosis</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase, γ-Glutamyl transferase</strong></td>
<td>Overproduction and release in blood - Hepatobiliary injury and cholestasis</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>Decreased synthesis - Hepatic dysfunction</td>
</tr>
<tr>
<td><strong>Total protein, Prothrombin time</strong></td>
<td>Decreased synthetic capacity - Hepatic dysfunction</td>
</tr>
<tr>
<td><strong>Glutathione-S-transferase</strong></td>
<td>Readily released from hepatocytes in response to injury - Early hepatocyte injury and necrosis</td>
</tr>
<tr>
<td><strong>Total bilirubin</strong></td>
<td>Decreased hepatic clearance - Hepatobiliary injury and cholestasis</td>
</tr>
<tr>
<td><strong>Urine bilirubin</strong></td>
<td>Leakage of conjugated bilirubin out of the hepatocytes into urine - Hepatobiliary disease</td>
</tr>
<tr>
<td><strong>Urobilinogen</strong></td>
<td>An increase in unconjugated bilirubin, due to increased breakdown of RBCs, which undergoes conjugation, excretion in bile and metabolism to urobilinogen – these are responsible for Hepatocellular dysfunction</td>
</tr>
<tr>
<td><strong>Bile acids</strong></td>
<td>Regurgitation into blood along with conjugated bilirubin - Hepatobiliary disease</td>
</tr>
<tr>
<td><strong>Purine nucleoside phosphorylase</strong></td>
<td>Released into hepatic sinusoids with necrosis - Hepatocellular necrosis</td>
</tr>
<tr>
<td><strong>Paraoxonase 1</strong></td>
<td>Reduced hepatic synthesis and secretion - Hepatocellular necrosis</td>
</tr>
</tbody>
</table>
1.5. KIDNEY AND NEPHROTOTOXICITY

The paired kidneys are bean-shaped organs situated on either side of the vertebral column between the T12 and L3 vertebrae. The right kidney located slightly lower than the left. An adult kidney is generally 10-12 cm long, 5-7 cm wide and 3 cm thick and weight 135-150 g. Kidneys are supported, protected and surrounded by the fat and connective tissue. Kidney is invested by a fibrous inner layer of dense collagen fibres from to superficial, which is known as the renal capsule. Kidney and its vessels are embedded in a mass of adipose tissue, known as adipose capsule, or perirenal fat. The thick outer layer of kidney is renal fascia, which attach the kidneys to adjacent structures [44, 46].

A frontal section of kidney discloses two distinct regions renal cortex (a superficial, light red area) and renal medulla (a deep, darker reddish-brown inner region). Renal cortex and renal pyramids of the renal medulla compose the functional portion of the kidney - parenchyma. Nephrons (about 1 million) are the functional unit of kidney presents within the parenchyma. Although the kidneys constitute with less than 0.5% of total body mass, but they receive 20–25% of the resting cardiac output. Renal blood flow in adults is about 1200 ml/min through both kidneys [44].

1.5.1. Overview of Kidney Function

The kidneys are involved in the major work of the urinary system and execute the following specific functions [44, 47].

- Maintaining the accurate osmolarity of body fluids, principally through regulating H₂O balance, and regulation of blood volume.
• Regulation of blood pH by excreting a variable amount of hydrogen ions (H\(^+\)) in urine and conserve bicarbonate ions (HCO\(_3^-\)).

• Regulation of the quantity and concentration of most extracellular fluid (ECF) ions, and to regulate blood ionic composition, by regulating the level of several ions like sodium (Na\(^+\)), chloride (Cl\(^-\)), potassium (K\(^+\)), calcium (Ca\(^{2+}\)), H\(^+\), HCO\(_3^-\), phosphate (HPO\(_4^{3-}\)), sulfate (SO\(_4^{2-}\)), and magnesium (Mg\(^{2+}\)).

• Maintenance of plasma volume level, which is vital in the long-term regulation of arterial blood pressure.

• Elimination of end products (wastes) of metabolism, such as urea, uric acids, creatinine, bilirubin, and hormone metabolites.

• Excretion of wastes and foreign compounds, like drugs, food additives, pesticides, and other exogenous nonnutritive materials.

• Production of hormone, like erythropoietin that stimulates red blood cell production and rennin that activates a chain reaction important in salt conservation by the kidneys.

• Conversion of vitamin D into its active form.

1.5.2. Nephrotoxicity

Nephrotoxicity can be explained as renal disease or dysfunction that causes from a direct or indirect exposure to medicines, and chemicals of environment and industry. It is well known that toxic nephropathies are not restricted to a single type of renal injury. Several chemicals target one distinct anatomical part of kidney and may affect only a particular cell type. It has become gradually more noticeable that a large
number of chemicals may adversely affect on one or more of the anatomical elements of the kidney, including glomerulus, proximal, intermediate, distal tubules, and medullary, endothelial, urothelial cells. Even though few of these cell types such as the proximal tubular cells have a distinct capability to repair damaged parts, but others like glomerular epithelium and "type 1" medullary interstitial cells do not have such ability. This phenomenon can affect the result of the chemical insult during any renal injury [65].

1.5.2.1. Transporters and nephrotoxicity

A number of physiologic factors play a role in determining the extent of chemical/drug induced nephrotoxicity. Transport systems that exist in the kidney play a key role in this regard. Toxic acute tubular necrosis is often connected with enhanced cellular uptake of nephrotoxic compounds. A large number of drug transport proteins have been recognized and have found to be expressed in renal proximal tubules. These transporters, in association with relatively high renal blood flow, influence the kidney to increased toxic susceptibility. Recent studies have shown that organic anion and cation transporter family, organic anion-transporting polypeptide family, type I sodium-phosphate transporters and ATP-dependent organic ion transporters such as MDR1/P-glycoprotein ABCB and the multidrug resistance-associated protein family ABCC may involve in such action. In addition, peptide transporter family, multi drug and toxin extrusion transporters have been reported to either transport or interact with several drugs. Nephrotoxicity emerges to be proportional to the final drug concentration, though the intrinsic characteristics of the drug like reactivity of drugs in intracellular targets are also important. Knowledge of these concepts and understanding the function of such transporters is important for the
prevention of iatrogenic kidney injury, particularly in patients taking potentially nephrotoxic agents [66, 67].

1.5.2.2. Drug induced nephrotoxicity

The incidence of drug-induced nephrotoxicity has been increasing in an alarming rate with the increasing uses of a number of drugs like NSAIDs, antibiotics, angiotensin converting enzyme inhibitors (ACEI) etc. A research in India showed that drug-induced acute renal failure (ARF) accounted for 20% of all ARF, of which aminoglycosides responsible for 40% of total cases [68]. Table 1.4 is the list of different nephrotoxic drugs and diseases caused by them [68, 69].

Table 1.4: List of some common drugs responsible for nephrotoxicity

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Pathogenesis</th>
<th>Mechanism/Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSAIDs, ACEI, cyclosporine, nerepinephrine, diuretics, interleukin-2, amphotericin-B</td>
<td>Pre-renal and functional failure</td>
<td>Due to reduced blood delivery</td>
</tr>
<tr>
<td>Aminoglycosides, NSAIDs, cephalosporins, amphotericin-B, rifampin, pentamidine, cisplatin, cephalothin, cyclosporine</td>
<td>Acute tubular necrosis</td>
<td>May be the consequence as in rhabdomyolysis or by the synergistic toxicity of two drugs</td>
</tr>
<tr>
<td>NSAIDs, allopurinol, sulfonamides, thiazides, methicillin, ampicillin</td>
<td>Acute intestinal nephritis</td>
<td>Systemic manifestation of hypersensitivity reaction</td>
</tr>
<tr>
<td>Drug Class</td>
<td>Diagnosis/Condition</td>
<td>Cause</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Methysergide, hydralazine, methyldopa</td>
<td>Retroperitoneal fibrosis</td>
<td>Unexplained uremia, polyuria are responsible for such condition</td>
</tr>
<tr>
<td>Penicillin G, ampicillin, sulfonamides, thiazides, metolazone</td>
<td>Hypersensitivity angiitis</td>
<td>Connected with vasculitis of small vessels can cause acute renal failure, extra renal manifestation</td>
</tr>
<tr>
<td>Mitomycin-C, cyclosporine, contraceptives, 5-fluorouracil, quinine, cocaine, ticlopidine, clopidogrel</td>
<td>Thrombotic microangiopathy/haemolytic uraemic syndrome</td>
<td>Connected with haemolytic anaemia, thrombocytopenia, renal dysfunction, Disease of CNS.</td>
</tr>
<tr>
<td>Gold, heroin, captopril, NSAIDs, D- penicillamine, interferon-alpha,</td>
<td>Isolated proteinuria with nephritic syndrome</td>
<td>Associated with edema, proteinuria with hypoalbuminuria</td>
</tr>
<tr>
<td>Heroine</td>
<td>Chronic glomerulopathy</td>
<td>Symptoms of uraemia with occasional fluid overload states and hypertension</td>
</tr>
<tr>
<td>NSAIDs, thiazides, lithium,</td>
<td>Chronic tubulointerstitial disease</td>
<td>Renal insufficiency develops slowly</td>
</tr>
<tr>
<td>Sulfadiazine, methotrexate, methoxyflurane, acyclovir, indinavir, nelfinavir, acetazolamide, triamterine</td>
<td>Drug induced crystalluria</td>
<td>Outflow tract obstruction due to crystal formation in tubules or ureter, retroperitoneal fibrosis</td>
</tr>
</tbody>
</table>
1.5.3. Mechanism of Nephrotoxicity

Most drugs found to exert toxic effects on kidney by one or more common pathogenic mechanisms which lead to nephrotoxicity. Some of these include altered intraglomerular hemodynamics, hepatocellular inflammation, crystal nephropathy, tubular cell toxicity, rhabdomyolysis, and thrombotic microangiopathy [69].

*Altered intra-glomerular hemodynamics:* The kidney regulates intraglomerular pressure by altering the afferent and efferent arterial tone to preserve glomerular filtration rate (GFR) and urine output. NSAIDs, ACEIs, angiotensin receptor blockers can alter the kidney function (regulation of glomerular pressure and GFR), while calcineurin inhibitors (e.g., cyclosporine, tacrolimus) can produce dose-dependent vasoconstriction of the afferent arterioles, leading to renal impairment [69, 70].

*Crystal Nephropathy:* Renal impairment may be caused by the use of drugs like ampicillin, ciprofloxacin, sulfonamides, acyclovir, foscarnet, indinavir, methotrexate that produce insoluble urine crystals, which precipitate usually within the distal tubular lumen, obstructing urine flow and eliciting an interstitial reaction [69, 70].

*Rhabdomyolysis:* In this syndrome, skeletal muscle injury leads to lysis of the myocyte, cause release of intracellular contents including myoglobin and creatine kinase into the plasma. Myoglobin causes renal injury secondary to direct toxicity, tubular obstruction, and alterations in GFR. Drugs like statins, abuse of cocaine, heroin, ketamine, methadone, and methamphetamine may initiate rhabdomyolysis directly, or indirectly by predisposing the myocyte to injury [69, 70].
**Thrombotic microangiopathy:** Organ damage results by the platelet thrombi in the microcirculation, as in thrombotic thrombocytopenic purpura. Mechanisms include an immune-mediated reaction or drug (quinine and antiplatelet agents like clopidogrel, ticlopidine, mitomycin-C) mediated direct endothelial toxicity [69, 70].

**Tubular cell toxicity:** Renal tubular cells are susceptible to the toxic effects of drugs like aminoglycosides, amphotericin B, antiretrovirals, cidofovir, tenofovir, cisplatin. These agents cause tubular cell toxicity through interfering mitochondrial function, impairing tubular transport, increase formation of free radicals or oxidative stress. Aminoglycosides can stimulate the calcium sensing receptor on the apical membrane and leads to cell signalling and cell death. Amphotericin B is responsible for altered cell permeability and causes toxicity by directly causing afferent arteriolar vasoconstriction [69, 70].

**Inflammation:** Inflammatory modification in the glomerulus, renal tubular cells, and the surrounding interstitium caused by drugs can leads to fibrosis and renal scarring. Glomerulonephritis is an inflammatory disease caused by immune mechanisms primarily and is often connected with proteinuria in the nephritic range. Gold therapy, hydralazine, interferon-α, lithium, NSAIDs, propylthiouracil, and pamidronate are responsible for such condition. Acute interstitial nephritis is another inflammatory disease resulted from an allergic response to a suspected drug like allopurinol, beta lactams antibiotics, acyclovir, indinavir, NSAIDs, phenytoin, omeprazole and ranitidine. Chronic interstitial nephritis is less common than acute interstitial nephritis caused by drugs like calcineurin inhibitors, aristocholic acid, and lithium) [69, 70]. Figure 1.8 discusses mechanism of drug induced nephrotoxicity.
Figure 1.8. Mechanism of drug induced nephrotoxicity

Drugs causes nephrotoxicity by following mechanism:
- Alter the kidney function
- Vasoconstriction of the afferent arterioles
- Impairing mitochondrial function
- Interfering with tubular transport
- Generation of free radicals
- Altered cell permeability
- Inflammatory changes in kidney
- Immune-mediated reaction
- Direct endothelial toxicity
- Obstructing urine flow and eliciting an interstitial reaction
1.6. HELMINTHS AND INTESTINAL HELMINTH INFECTIONS

Helminths are parasitic worms that have plagued humans since earlier than the era of our first recorded history. More than two billion people are infected with parasitic helminths. Although infections caused by helminths are generally less or not fatal, they are connected with high rates of morbidity, with chronic infection frequently leading to anaemia and malnourishment. If the helminth infections left untreated, this causes multi-year, chronic inflammatory disorders with both delayed and concurrent-onset pathology to the afflicted human host [71].

1.6.1. Helminths and Types of Helminths

The helminths are worm-like parasites, relatively large (> 1 mm long) or very large (> 1 meter long) in size. They exit as both hermaphroditic and bisexual species, with well-developed organ systems and most are active feeders. The body of helminths is either cylindrical or covered with cuticle (roundworms) or flattened and enclosed with plasma membrane (flatworms). Depending upon their general external shape and the host organ they live, helminths can be classified clinically into two major groups [72],

(a) Platyhelminthes or flatworms and

(b) Nematoda or roundworms.

Platyhelminthes are also known as flatworms, and include the flukes (trematodes), such as the schistosomes, and the cestodes (tapeworms), such as the pork tapeworm. Adult flukes recognised as leaf-shaped flatworms, with prominent oral and ventral suckers that help maintain position in situ. Majority of flukes are hermaphroditic, but the blood flukes are bisexual. The life-cycle of flukes includes
intermediate host snail. Tapeworms are the adult, elongated, segmented, hermaphroditic flatworms that reside in the intestinal lumen. Larval forms are cystic or solid, reside in extraintestinal tissues [71, 72, 73].

The nematodes are also known as roundworms, these include the major intestinal worms (soil-transmitted helminths) and the filarial worms that responsible for lymphatic filariasis and onchocerciasis. Adult and larval roundworms are cylindrical and bisexual worms inhabit intestinal and extraintestinal sites [71, 73].

1.6.2. Mode of Transmission and Factors Affecting Susceptibility

The mode of transmission of infection differs with the type of worm; mode of transmission includes ingestion of eggs or larvae, penetration by larvae, vectors bite, or taking the meat of intermediate hosts. Many of these helminth infections are asymptomatic, and the pathologic manifestations depend on the physical characteristic, activity, and metabolism of the worms. Transmission of helminths to humans involves a number of pathways [72],

- Accidental ingestion of infective eggs of Ascaris, Echinococcus, Enterobius, Trichuris or larvae of few hookworms.

- Penetration of skin actively (like, hookworms, schistosomes etc.).

- Infective parasite transmitted from the intermediate vector when vector bites the host to get a blood meal (e.g. the arthropod vectors of filarial worms).

- Human eats that intermediate host that contained the larvae of parasite (e.g. tapeworms in meat and fish, Trichinella in meat).
Susceptibility of parasite infection also depends on a number of factors like standards of hygiene (as urine or feces often contain eggs and larvae), on the climate (which may favor endurance of infective stages), on the ways of food preparation, prepared, and on the exposure time to insect vectors [72].

Mainly two factors are involved in the susceptibility of parasite infection like [72].

(A) Host factors:

- Behavioral factor like hygiene and food, and exposure.
- Host's nonspecific defense mechanisms.
- Drug therapy like irradiation and immunosuppressant drugs

These factors may increase susceptibility to helminth infection.

(B) Parasite factors:

- Ability of parasites to evade the host's defenses.
- Physical characteristics like size, length, mobility large and mobility of helminths.

1.6.3. Intestinal Helminths

More than 342 species of helminths have been found in association with humans, among them nearly 197 species would be believed as primarily inhabitants of the gastrointestinal (GI) tract [74, 75].

Intestinal helminths are widespread and common in the developing world; current estimates indicate that one-third of the world’s population is infected by
helminth infection. Around 20 species are responsible for different disease, of which 6 species like Enterobius, Ascaris, Necator, Ancylostoma, Trichuris and Strongyloides together responsible for diseases caused in over half of the world’s population [74, 75].

Many helminth infection are considered as accidental infections from other species that are normal hosts or perhaps common in some communities. Most of the GI helminths infections are light and asymptomatic; they do not cause significant morbidity and mortality, but have a prominent impact on nutrition, pregnancy outcome, growth, physical fitness, cognitive functions and anaemia in infants, children and adults. Many factors like social, behavioural and genetic determine the susceptibility of infections in an individual. Age also considered as key factor for GI Helminth infection vary between species; there is an increase in hookworm intensity with age, but high intensity in childhood for Ascaris and Trichuris infections [74, 75].

The complexity of the immune response of host to helminth infection is rivalled only by the evasive mechanisms of helminth parasites have progress to make sure their survival [76]. These include strategies intended to

- Evade induction or perpetuation of the host immune response (e.g. sequestration, molecular mimicry or disguise, antigen or antibody shedding).
- Downregulate the immune response of host to parasite antigens.
- Combat against the specific components of host immune attack like secretion of protease inhibitors, antioxidants and other enzymes.

Figure 1.9 includes some of the common intestinal worm and their mode of infection.
1.6.4. Protective Immune Mechanisms in Helminth Infection

The immune system in mammals is a carefully tuned orchestra consisting of a variety of cells, producing humoral (antibody) and cellular immunity. Central to most of the immune functions are T helper (Th) cells, which play a critical role in instructing and controlling the various immune response players against invading pathogens. Based on the type of cytokinin produced these T helper cells can be classified in different types such as T_{H1} and T_{H2} cells [77].

The protective immune response against many helminth parasites generally referred as T_{H2} (T helper 2) response. T_{H2} cells orchestrate the immune response mainly through the production of several cytokines in the lymph nodes and periphery. T_{H2}-type responses are naturally considered by the increase levels of interleukin-4 (IL-4) and cytokines like IL-5, IL-9, IL-13 and IL-21, activation and expansion of CD4^+
$T_h^2$ cells, plasma cells secreting immunoglobulin E (IgE), eosinophils, mast cells and basophils, all of which can generate several types of $T_h^2$-type cytokine. IL-5 triggers eosinophilia, and in combination with IL-4, IL-9, and IL-13, and crosslinking of FceRIIs (high-affinity Fc receptors for IgE) results improved mast-cell and basophil development and release of mediators. IL-4 and IL-13 causes amplified smooth muscle cell contractility, increased intestinal permeability, increased goblet-cell mucous secretion and enhance mast-cell-derived mediators. Together, these effects can cause to the ‘weep and sweep’ response to intestinal helminths. IL-4 can induce class switching in B cells in conjunction with other signals that leads to IgE production. IL-4, IL-13 and IL-21 can trigger the development of alternatively activated macrophages, leading to up-regulation of arginase-1 expression, though in some cases this might lead to fibrosis, as in chronic schistosomiasis. IL-17 linked cytokine IL-25 is also connected with the $T_h^2$-type response and can endorse $T_h^2$-cell differentiation and nematode parasite expulsion, though the nature of IL-25 is remains unclear. In comparison, interferon-$\gamma$ dominant $T_h^1$-type responses are classically evoked by microbial (bacteria and viruses) infections, and are related with increase in $T_h^1$ cells, cytotoxic CD8$^+$T cells, neutrophils and macrophages count. Initially IL-10 was characterized as a $T_h^2$-type cytokine, but resent researchers suggested that this cytokine is also produced by $T_h^1$ cells and regulatory T cells $in$ $vivo$, and can down-regulate both $T_h^1$-type and $T_h^2$-type responses. A number of cytokines like IL-4, IL-13, IL-21 and IL-25 that are preferentially expressed during the $T_h^2$-type response can also down regulate $T_h^1$-type and $T_h^{17}$-type responses and their associated inflammation [78].
Figure 1.10: Protective TH2-type response during intestinal nematode infection
[IL, interleukin; AAMs, alternatively activated macrophages; T\(_2\), T helper 2]
Development of granuloma in helminth infections are traditionally connected with localized T\(_H1\)-type inflammatory responses that develop around a nidus, such as an invading microorganism or parasite. For example, in acute schistosome infection causes initial T\(_H1\)-type response that targets adult parasites, but typically transitions to a T\(_H2\)-type response after the parasite’s eggs are produced. Innate immune effector cells are vital for both the initiation and effector phases of T\(_H2\)-type immune responses in helminth infections. CD4\(^+\) T\(_H2\) effector cells causes up regulation of innate effector-cell response primarily through the secretion of cytokines promote expansion of the T\(_H2\) effector-cell population. These results fine-tune targeted effector functions against the invading helminth parasite [75]. Figure 1.10 represents a simplified protective T\(_H2\)-type response during intestinal nematode infection.