Chapter 1

"Before you start some work, always ask yourself three questions - Why am I doing it, What the results might be and Will I be successful. Only when you think deeply and find satisfactory answers to these questions, go ahead."

Chanakya
1 INTRODUCTION

1.1 GENERAL INTRODUCTION

The knowledge of drugs goes back to primeval times. The evolution of medicine as a process of trial and error has marked man's tireless crusade against the ravages of pestilence and sickness. Disease has always been a great topic of concern in human society. From prayers and spells to the birth of medicine as a rational science, man has been able to develop all sorts of medical treatments to combat against different illnesses and ailments, because as the Chinese proverb has it, 'life is worth more than a thousand gold pieces'\(^1\). Thus remedy, sickness and healing constitute the core elements of a society’s medical institution. If disease, injury, and physiological process are the biologically based substrates of medicine, then sickness (the socially constructed expression of a subjective state of illness) and healing (the efforts, procedures, and medicines aimed at neutralizing illness) are its social and cultural realization\(^2\). Medicines are as a result, the core of health care, cure for diseases (such as antibiotics), reliever of symptoms (such as analgesics), are preventive (such as anti-hypertension drugs) or substitute for endogenous compounds (such as insulin)\(^3\).

The historical analysis of medicine reveals that man has always been in search of cures for a variety of ailments that caused physical, physiological and psychological discomfort and consequently it is assumed that the search for medicine began with the advent of humans on this earth\(^4\). Then again it has been reported that some animals, including insects, amphibians, mammals and birds, protect themselves against enemies by exploiting the chemical defense mechanisms of plants\(^5\), furthermore, it is a common knowledge that animals (including carnivores) often feed
on certain plants, grasses or berries when they are sick, which has even helped in isolating novel compounds for human diseases$^{6-14}$, consequently, it can be conceived that humans may have learned to use plants and plant-derived products as remedies for various ailments, by taking cues from animals and/or through trial and error, leading to the discovery of various remedies$^{6,12,13}$. So the history of natural medicine might be much older than human civilization.

At present effective medical treatments are available for many ailments. Recent discoveries include drugs that treat hypertension, ulcers, and liver disorders. For other devastating diseases, such as diabetes, cancer, AIDS, stroke, and alzheimer disease, only inadequate therapy is available; new approaches to treat these disorders are sought intensively by researchers. What's more, in future, new infectious agents may be expected to emerge as significant threats, like the human immunodeficiency virus (HIV) that still possess a challenge to us. Because of enormity of such challenges, we continue to seek to improve the strategies by which we discover new drugs$^{15}$.

1.2 HERBS

Herbs are defined in several ways depending on the context, which the world is used. In the field of medicine, they are most accurately defined as crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health. Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluid extracts, extracts, or the like, are known as Phytomedicinals$^{16}$. Herbs are used as medicine by about 80% of the world population, mainly in the developing countries, for primary healthcare because of better cultural acceptability, better compatibility with the human body and lesser side effects$^{17}$. India is one of the countries in the world today where
ancient system of medicine, such as Ayurveda, Siddha, Unani, Tribal medicine and Naturopathy have been in practice for several years\textsuperscript{18-19}.

Medicinal plants are not only used for primary health care and not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used\textsuperscript{20}. In western world also, the use of herbal medicines is steadily growing with approximately 40 percent of population reporting use of herb to treat medical illnesses in 2004\textsuperscript{21}. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine\textsuperscript{22}.

The major hindrance in the amalgamation of herbal medicines into modern medical practices is the lack of scientific and clinical data and better understanding of efficacy and safety of the herbal products. To ensure the quality, safety and efficacy of its products and practices, standardization is of vital importance. Most of the herbal products do not have drug regulatory approval to demonstrate their safety and efficacy. The traditional use can provide valuable clues for the selection, preparation and indications for use of herbal formulations, as efficacy has been established by the common use. The historical use provides the source to study the specific plant species with potential to be used in a particular disease. A systematic approach through experimental and clinical validation of efficacy is required for a plant identified for traditional medicine, as is done in modern medicine\textsuperscript{23}.
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1.3 MEDICINAL PLANTS IN INDIA

Medicinal plants are a source of great economic value in the Indian subcontinent\textsuperscript{24} and India has one of the richest plant medical traditions in the world\textsuperscript{25}. India has a tradition that is of remarkable contemporary relevance for ensuring health security to the millions. Nature has bestowed on India a rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity\textsuperscript{24}. India has 2.4% of world's area with 8% of global biodiversity. It is one of the 12 mega-diversity hot-spot regions of the world\textsuperscript{25}. There are about 45,000 plant species in India, with concentrated hot spots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island\textsuperscript{26}. It is difficult to estimate the number of medicinal and aromatic plants present worldwide; the fact remains true that India with rich biodiversity ranks first in percent flora, which contains active medicinal ingredient and 20-44\% of all the plants found in India are used for medicinal purpose\textsuperscript{24}.

Around 70 percent of India's medicinal plants are found in the tropical areas mostly in the various forest types spread across the Western and Eastern Ghats, the Vindhyas, Chotanagpur plateau, Aravalis, the Tarai region in the foothills of Himalayas and the North-East. Less than 30 percent of the medicinal plants are found in the temperate and alpine areas of higher altitudes which include species of high medicinal value. A small number of medicinal plants are also found in aquatic habitats and mangroves (Table 1)\textsuperscript{25}. 
Table 1: Medicinal plants species diversity and representative species of biogeographic zones of India

<table>
<thead>
<tr>
<th>Bio-geographic region</th>
<th>Estimated No. Of Medicinal Plants</th>
<th>Example of some typical medicinal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desert</td>
<td>500</td>
<td><em>Convolvulus microphyllus</em> Scib ex Spreng., <em>Tecomella undulata</em> (Sm), Seem., <em>Citrullus colocynthis</em> (L.), Schrader and <em>Cressa cretica</em> L.</td>
</tr>
<tr>
<td>Semi-Arid</td>
<td>1,000</td>
<td><em>Commiphora wightii</em> (Arn.) Bhandari, <em>Caesalpinia bonduc</em> (L.), Roxb., <em>Balanites aegyptiaca</em> (L.), Delilie and <em>Tribulus rajasthanensis</em> Bhandari &amp; Sharma</td>
</tr>
<tr>
<td>Western Ghats</td>
<td>2,000</td>
<td><em>Myristica malabarica</em> Lam., <em>Garcinia indica</em> (Thou.) Choisy, <em>Utleria salicifolia</em> Bedd. and <em>Vateria indica</em> L.</td>
</tr>
<tr>
<td>Deccan Deccan Peninsula</td>
<td>3,000</td>
<td><em>Pterocarpus santalinus</em> L.f., <em>Decalepis hamili</em> Wight &amp; Arn., <em>Terminalia pallida</em> Brandis and <em>Shorea tumbugaia</em> Roxb.</td>
</tr>
<tr>
<td>Gangetic Plain</td>
<td>1,000</td>
<td><em>Holarrhena pubescens</em> (Euch-Ham.) Wall. ex DC., <em>Mallotus philippensis</em> (Lam.) Muell-Arg., <em>Pluchea lanceolata</em> C.B. Clarke and <em>Peganum harmal</em> L.</td>
</tr>
<tr>
<td>North-East India</td>
<td>2,000</td>
<td><em>Aquilaria malaccensis</em> Lam., <em>Smilax glabra</em> Roxb., <em>Ambroma augusts</em> (L.) L.f. and <em>Hydnocarpus kurzii</em> (King) Warb.</td>
</tr>
<tr>
<td>Islands</td>
<td>1,000</td>
<td><em>Calophyllum inophyllum</em> L., <em>Adenanthera pavonina</em> L., <em>Barringtonia asiatica</em> (L.), Kurz <em>Aisandra butyracea</em> (Roxb), Baehni</td>
</tr>
<tr>
<td>Coasts</td>
<td>500</td>
<td><em>Rhizophora mucronata</em> Lam., <em>Acanthus ilicifolius</em> L.,</td>
</tr>
</tbody>
</table>
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Fig. 1: Habit-wise distribution of medicinal plants

Fig. 2: Distribution of medicinal plants by families
Analysis of habit-wise distribution of medicinal plants indicates that a little more than one-third is a tree. Herbs form around one-third of the population. Shrubs and climbers form a little more than one third of the total (Fig. 1); the known medicinal species largely occur amongst angiosperms and to a lesser extent in groups like algae, fungi, lichens, bryophytes, pteridophytes, and gymnosperms; of the 386 families and 2,200 genera in which medicinal plants are recorded, the families Asteraceae, Euphorbiaceae, Lamiaceae, Fabaceae, Rubiaceae, Poaceae, Acanthaceae, Rosaceae and Apiaceae share the larger proportion of medicinal plant species, with the highest number of species (419) falling under Asteraceae (Fig. 2)\textsuperscript{26,27}.

Utilization of plants for medicinal purposes in India has been documented long back in ancient literature. However, organized studies in this direction were initiated in 1956 and off late such studies are gaining recognition and popularity due to loss of traditional knowledge and declining plant population\textsuperscript{26,28}. Plants that are recognized for their medicinal use in India, can be placed into the following three categories: (i) plants of codified knowledge (over 1500), used in organized systems of medicine like Ayurveda, Unani and Siddha, (ii) plants of empirical knowledge (3000), used in ethno medicine or folk medicine based on oral (undocumented) information being passed from generation to generation and (iii) plants of scientific knowledge (700), which have been investigated pharmacologically and chemically, and their active principles are used in modern medicine or are providing valuable leads for partial or total synthesis of new drugs\textsuperscript{29,30}. Thus India has a wealth of information in terms of the number of unique species documented thus far for their medicinal use and also in terms of the tremendous depth of traditional knowledge about such uses for human & livestock health and also for agriculture\textsuperscript{24-28}. 
India has the second largest tribal population in the world after Africa and these tribal people mostly depend on forests for their livelihood. Many tribal communities in India still practice use of their traditional knowledge to cure a variety of diseases and ailments. Over 4786 ecosystem specific species of plants are used by ethnic communalities for human and veterinary health care, across the various ecosystems from Ladakh in the trans-Himalayas to the southern coastal tip of Kanyakumari and from the deserts of Rajasthan and Kachch to the hills of the Northeast. Plants and their parts are not only used as food and medicine but also used in various tribal rituals that are a part of their social and religious life.

1.4 HERBAL MEDICINE: RESEARCH, DEVELOPMENT AND MARKET

There is a great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lesser costs. Since herbal medicines are prepared from materials of plant origin they are prone to contamination, deterioration and variation in composition. This gives rise to inferior quality of herbal products with little or no therapeutic efficacy. But the knowledge and experimental data base of Ayurvedic medicine can provide new functional leads to reduce time, money and toxicity - the three main hurdles in drug development. These medicines are effectively tested for thousands of years on people.

It is rightly said that ‘laboratories to clinics’ becomes ‘clinics to laboratories’ - a true reverse pharmacology approach.

Currently, with over 4000,000 registered Ayurvedic practitioners, the Government of India has formal structures to regulate quality, safety, efficacy and practice of herbal medicine (National policy on Indian systems of Medicine and Homeopathy - 2002). The turnover of herbal medicines in India as over - the counter
products, ethical and classical formulations and have remedies of Ayurveda, Unani and Siddha systems of medicine is about $1 billion with a meager export of $80 million. 80% of its exports to the developed countries are of crude drugs and not finished formulations leading to low revenue for the country. Allium salivum, Aloe barbadensis and Panax species are three of the 10 most widely selling herbal medicines in developed countries which are imported from India. India is also the largest grower of Psyllium (Plantago ovata), Senna (Cassia Senna) and Castor (Ricinus communis) plant. Herbal preparation are being marketed as nutraceuticals or health foods and many pharma and biotech companies market pure compounds of natural origin like lovastatin (a lipid lowering agent from red rice yeast), docisahexaenoic acid (a cardiovascular stimulant from algae), stevols, curcumin (from plants), etc.

It is estimated that nearly three fourths of the herbal drugs used worldwide were discovered following leads from local medicine. According to WHO about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated from plants. Investigations of plants used in traditional and modern medicine in China serve as a source of inspiration and as models for the synthesis of new drugs with better therapeutic, chemical or physical properties than the original compounds. The market for herbal medicine is estimated to be expanding at 25% annually. Sales of medicinal plants based drugs have grown by nearly 30% in India in the year 1996-2006, the highest rate of the growth in the world. Lilly Research Laboratories markets several million dollars’ worth of vincristine and vinblastine – the periwinkle derivatives used to treat childhood leukaemia and Hodgkin’s disease. The US National Cancer Institute regularly earmarks large appropriations to screen 50,000 natural substances for
activity against cancer cell lines and the AIDS virus. China, Germany, India and Japan among others are also screening wild species for new drugs\textsuperscript{33-40}.

The diversity of medical uses of plant is at times daunting for a new entrant to the field. But for a multidisciplinary research and development network, the options of research approach provide deep motivation for identification of new pharmacophores. Besides expanding the herbal therapeutic and preventive armamentarium, new pharmacophores may help to evolve new targets of drug action as well as a possibility for combinatorial chemistry on the novel pharmacophores. For example, curcumin has been a target molecule for a significant endeavour for a large number of combinatorial compounds.

The Council of Scientific and Industrial Research (CSIR) in India has initiated sizeable and meaningful efforts for the development of herbal-based formulations for diabetes, arthritis and hepatitis by a national network programme (Fig. 3). The industry, academia and the government research laboratories work in close collaboration. Interesting and novel activities have been detected with the selected plants and some of the active ingredients of therapeutically demonstrable effects were isolated\textsuperscript{34,35,41}.

Ayurveda is the most ancient health care system and is practiced widely in India, Srilanka and other countries. Atharveda (around 1200 BC), Charak Samhita and Sushrut Samhita (100 - 500 BC) are the main classics that given detailed descriptions of over 700 herbs. Researches on pharmacognosy, chemistry, pharmacology and clinical therapeutics have been carried out on Ayurvedic medicinal plants and many of the major pharmaceutical corporations have renewed their strategies in favour of natural products drug discovery.
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Approach to Natural Product Drug Discovery.

Collection of Natural Products
Identification of Natural Product Reportedly In Use.
Transport of Materials To The Research Laboratory.
Storage
Preparation of Extracts
Toxicity Studies of The Extract In Animals
Evaluation of Therapeutic Efficacy of Extract (In vitro and In vivo).
Characterization of The Extract Having More Activity
Structural Elucidation of The Bioactive Molecule
Further Purification of The Active Molecule
Synthesis of The Bioactive Compound In Large Scale

Fig. 3: Approach for drug development from natural sources
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Numerous drugs have entered the International Pharmacopoeia through the study of ethnopharmacology and traditional medicine. The R & D thrust in the pharmaceutical sector is focused on development of new innovative / indigenous plant- based drugs through investigation of leads from the traditional system of medicine. The World Health Organization (WHO) has recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines. It is necessary to develop methods for rapid precise and accurate identification and estimation of active constituents in order to bring out consistency of important constituents in the formulations. The Pharmaceutical Research and Development Committee (PRDC), under the Ministry of Chemicals, Government of India also underlines the importance of traditional medicines.

The basic uses of plants in medicine will continue in the future, as a source of therapeutic agents, and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes and food industries. Popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as increasing public costs in the daily maintenance of personal health and well-being. In the dual role as a source of healthcare and income, medicinal plants make an important contribution to the larger development process. The World Health Organization also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines35-46.
1.5 HERBAL FORMULATIONS

An important step in the development of herbal medicine is development of its formulations. Herbs and herbal extracts pose typical problems in the development of formulations such as hygroscopic nature, microbial contamination, stickiness, slipperiness, and stability. Hence, to get a proper formulation, it is necessary to tackle all these problems.47

The actual herbal drug, the nature and concentration of the extraction solvent and the manufacturing process all contribute to final herbal formulation. In the past few years, enormous efforts have been made to optimize and validate the manufacturing process of herbal extracts in order to achieve a reproducible composition of the pharmaceutically relevant constituent by means of a detailed quality assurance program.

Now a days it is possible to assure the quality of the herbal extracts in such a way that manufacturers can produce batch-to-batch consistency and as a result reproducible therapeutic effects.

Definition of herbal formulations:

Herbal formulations are the formulation prepared by plant extracts either of barks, leaves, stem or roots with suitable excipients. These extracts contain some of the active constituents, thus with suitable excipients or base different formulations are prepared and formulations are prepared and standardized with suitable methods.37 When two or more herbs are used in formulations, they are known as polyherbal formulations.
Limitations of Ancient Formulations:

The major reasons for not applying quality assurance techniques to herbal formulations are:

- In olden days Ayurvedic formulations were prepared by Vaidyas as and when necessary in fresh form for administration.
- Active constituents vary from species to species, geographic and seasonal variations. They were not manufactured for mass consumption.
- The contribution of drugs and dosage form differ from person to person depending upon intensity of diseases.
- Most of the Ayurvedic preparations have to be consumed with specific vehicles such as Ghee, Honey, Milk, Water etc. prior to consumption. In many cases even though medicine is the same but vehicles are different in different conditions.

Problems with Modern (Allopathic) Drugs:

- High cost and longtime taken in development of new drug.
- Toxicity – A new branch of medicine is termed iatrogenic diseases.
- Non-renewable source of basic raw materials. Most synthetic drug utilizes fossil resources like petrochemicals.
- Environmental pollution by the chemical industry.
- Inadequate, especially in management of certain chronic diseases.

Advantages of Plant-based Drugs:

- Long history of use and better patient tolerance as well as public acceptance.
- Renewable source.
- Cultivation and processing environmental friendly.
- Local availability, especially in developing countries.
- Plants constitute to be a major source of new lead generation.
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Today we are more concerned with life style diseases like depression, cancer and heart troubles caused by faulty nutrition and stress. Because these diseases have a mental or emotional component, there is a growing conviction that allopathy is largely unable to cure them, all of it offers is temporary relief from symptoms. Moreover modern medicine does not have a suitable answer for many conditions such as liver disorder and for chronic conditions such as asthma, arthritis, etc. and this leads to increase interest in herbal drugs. There is a need of alternative therapy, to cover a good health for all. Herbal therapy will be one of the best practices to overcome the illness. Since liver plays a vital role in the metabolism of various exogenous and endogenous compound. As a result of its continuous involvement is susceptible to toxic injuries caused by certain agents, and any damage to hepatic cells disturb body metabolism. Indiscriminate use of systemic agents like tetracycline, paracetamol, antitubercular drugs, oral contraceptives of hormonal origin, chemicals used as food preservatives and agrochemicals are threatening the integrity of the liver.

1.6 LIVER

Liver (Fig. 4) is the largest organ in the body, situated in the upper part of the abdominal cavity occupying the greater part of the hypochondriac region. It consists of four lobes, which divides into lobules. Lobule is the basic functional unit of the liver. The liver lobules are made up of columns of hepatic cells whose outlines are indistinct forming a syncytium.

The liver performs over 500 different chemical functions and affects nearly every physiological process of the body. More heat is produced by the liver than other organ in the body, which is very essential in maintenance of body temperature.
The liver is among the few internal human organs capable of natural regeneration of lost tissue; as little as 25% of remaining liver can regenerate into a whole liver again and it is a fastest regenerating tissue of the body.

Overeating is a common cause of liver malfunction because it creates excessive work for the liver and an overworked liver can't properly detoxify harmful substances. Chinese doctors have long considered the liver to be the most important organ in the body - they call the liver, the "General of the Army".

1.6.1 ANATOMY AND PHYSIOLOGY OF LIVER

Liver is a largest gland in the body, weighing between 1 to 2.5 kg situated in the right upper quadrant of the abdomen, just below the diaphragm. Its upper and anterior surface are smooth and curved to fit the under surface of the diaphragm; and posterior surface is irregular in outline. A thick capsule of connective tissue called Glisson's capsule covers the entire surface of the liver.
Fig. 5: Microscopic structure of the liver

A] A magnified transverse section of a liver lobule
B] Direction of the flow of blood and bile in a liver lobule
The liver is a multi-lobed organ, i.e., it has four distinct lobes, divided into a large right lobe and a smaller, wedge-shaped left lobe, the other two, the caudate and quadrate lobes.

The falciform ligament divides the two lobes of the liver. Each lobe is further divided into lobules that are approximately 2 mm high and 1 mm in circumference. These hepatic lobules (Fig. 5) are the functioning units of the liver, each of them have approximately 1 million lobules that consist of a hexagonal row of hepatic cells called “hepatocytes”. They secrete bile into the bile channels and also perform a variety of metabolic functions. Between each row of hepatocytes are small cavities called “sinusoids”, and each sinusoid is lined with Kupffer cells, phagocytic cells that remove amino acids, nutrients, sugar, old red blood cells, bacteria and debris from the blood that flows through the sinusoids. The main functions of the sinusoids are to destroy old or defective red blood cells, to remove bacteria and foreign particles from the blood and to detoxify toxins and other harmful substances.

Almost all blood that enters the liver via the portal tract originates from the gastrointestinal tract as well as from the spleen, pancreas and gallbladder. Total human liver blood flow represents approximately 25% of the cardiac output up to 1500 ml/min. Hepatic flow is subdivided in 25-30% for the hepatic artery (500 ml/min) and the major part for the portal vein (1000 ml/min). A second blood supply to the liver comes from the hepatic artery, branching directly from the celiac trunk and descending aorta. The portal vein supplies venous blood under low pressure conditions to the liver, while the hepatic artery supplies high-pressured arterial blood. Since the capillary bed of the gastrointestinal tract already extracts most O₂, portal venous blood has a low O₂ content. Blood from the hepatic artery on the other hand,
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originates directly from the aorta and is, therefore, saturated with O₂. Blood from both vessels joins in the capillary bed of the liver and leaves via central veins to the inferior vena cava just below the diaphragm.

1.6.2 FUNCTIONS OF THE LIVER

The liver is responsible for important functions, which includes

- It produces and secretes bile (stored in the gallbladder until needed) that is used to break down and digest fatty acids.
- It produces prothrombin and fibrinogen, both blood-clotting factors.
- It converts sugar into glycogen, which it stores until the muscles need energy and it is secreted into the blood stream as glucose.
- It synthesizes proteins and cholesterol and converts carbohydrates and proteins into fats, which are stored for later use.
- It produces blood proteins and enzymes needed for digestion and other bodily functions.
- It produces urea, while breaking down proteins, which it synthesizes from carbon dioxide and ammonia. It is eventually excreted by the kidneys.
- Liver also stores critical trace elements such as iron and copper, as well as vitamins A, D, and B₁₂.
- The liver is also responsible for detoxifying the body of poisonous substances. The liver synthesizes and transports bile pigments and bile salts that are needed for fat digestion. Bile is a complex mixture of bile salts, bile pigments, alkaline phosphatase, water and various lipids, include cholesterol, lecithin and bilirubin. Bile salts are produced by the metabolism of cholesterol, are involved in the absorption and metabolism of fat.
Bilirubin is the main bile pigment that is formed from the breakdown of hemoglobin liberated when red blood cells are broken down in the reticuloendothelial system. The break down haem travels to the liver, where it is secreted into the bile by the liver. Bilirubin production and excretion follow a specific pathway. When the reticuloendothelial system breaks down old red blood cells, bilirubin is one of the waste products. This “free bilirubin” is a lipid soluble form that must be made water soluble to be excreted.

The conjugation process in the liver converts the bilirubin from the fat soluble to a water soluble form. The liver also plays a major role in excreting cholesterol, hormones, and drugs from the body. The liver plays an important role in metabolizing nutrients such as carbohydrates, proteins and fats. Thus liver helps in metabolizing carbohydrates.

A) Metabolism

Liver is an organ that orchestrates the metabolism of fats, carbohydrates and proteins. It does this in conjunction with the circulatory system, the lymphatic system and the endocrine (hormone) system. A healthy liver is critical to proper protein, carbohydrate and fat metabolism.

Protein Metabolism: The liver produces all of the proteins except for the proteins synthesized by the immune system (called γ globulins or immunoglobulins). It does this by reassembling amino acids into protein. The main protein produced by the liver is called albumin. Normal albumin in the bloodstream is important for many physiologic functions. One of these functions involves the normal maintenance of fluid pressure in the arteries and veins. When the protein level falls below a certain point the fluid in these vessels can leak out and pool in the abdominal or thoracic cavities. This fluid is called ascites when it occurs in the abdominal cavity, and
called pleural effusion when it occurs in the thoracic cavity. Albumin also functions to "carry" other compounds through the bloodstream. These compounds include calcium, vitamins, hormones, fatty acids, many drugs and bilirubin. A consistent finding with liver disease is a low protein level (hypoproteinemia). This low level usually occurs only when the liver has been severely diseased for a prolonged period of time, because of the great reserve capacity of the liver to produce more albumins.

**Carbohydrate Metabolism:** Glucose is stored in hepatocytes in the form of glycogen. It is used as a reservoir during times when carbohydrate intake is low (fasting or starvation). The liver can also manufacture glucose from proteins or fats. In liver diseases the body can have a difficult time regulating the blood glucose level, usually leading to hypoglycemia (low blood glucose). This is one of the reasons why caloric intake is an important aspect of treatment.

**Lipid Metabolism:** The liver regulates fats (called fatty acids) in the bloodstream. It does this by converting excess amounts of carbohydrates and proteins into fatty acids. The liver also synthesizes cholesterol from this fat. Cholesterol is necessary for many functions, particularly the sex hormones and steroids like cortisone. Excess fatty acid accumulation in the hepatocytes is called lipidosis.

**B) Detoxification**

Drug metabolism is an important liver function. It is a complex process that occurs in the endoplasmic reticulum of the hepatocyte. Several phases are involved with this detoxification:

**Phase-I reaction:** In phase 1 reaction, oxidation or demethylation occurs, mediated by cytochrome P-450. A variety of oxidative phase 1 reactions are performed by the enzymes that make up the P-450 system. Found primarily in the liver but also in the gastrointestinal tract, kidneys, brain and other tissues. Cytochrome P-450
enzymes are composed of a unique apoprotein and a heme prosthetic group, which binds oxygen after electron-transfer reactions from NADPH, resulting in aliphatic and aromatic hydroxylation, O, N, or S dealkylation or dehalogenation. A typical reaction of this type generates a hydroxyl group, which can then participate in the phase 2 reactions. Each group of genes with 40 percent amino acid homology composes a family whose gene products (isoenzymes) may function in a similar fashion. For example, CYP3 is a family that contains an A subfamily and several genes, numbered 1, 2, and so forth. The primary enzyme for the metabolism of erythromycin in humans is CYP3A4.

**Phase II reaction:** After a phase 1 reaction, most compounds are still not very water-soluble and require further metabolism. In a typical phase 2 reaction, a large water-soluble polar group is attached to hydroxyl oxygen by glucuronidation or sulfation, forming ether or ester linkages. These are the sole steps required for the hepatic metabolism of some compounds. But for most, the phase 2 reaction is preceded or followed by phase 1 oxidation. Compounds requiring glucuronidation include acetaminophen, morphine, and furosemide, as well as bilirubin. Sulfation is as important as glucuronidation, particularly for the metabolism of steroid compounds and bile acids. There are several species of sulfotransferases with overlapping specificities, each employing 3-phosphoadenosine-5-phosphosulfate synthesized from ATP and sulfate ions. Although phase 2 reactions are usually accomplished without a detrimental effect, they can occasionally lead to toxic or carcinogenic by products.

**Bile Metabolism:** Bile is secreted by liver and drugs are eliminated in the bile, red blood cells are recirculated through the bile system and fats are absorbed from the intestines into the bloodstream only in the presence of bile.
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When red blood cells break down and are recycled they release bilirubin from their hemoglobin. The liver, along with spleen and bone marrow, recycle this bilirubin, salvaging some of the compounds (iron) and excreting the rest in the bile. Bilirubin, which is toxic, binds to albumin and is detoxified and excreted. This is eventually excreted into the intestines and broken down by intestinal bacteria into urobilinogen, where it imparts the dark color to stool. If this bilirubin cannot be excreted from the gallbladder (when there is an obstruction in the bile duct) there will be very light colored (acholic) stool. The excess amounts of bilirubin that build up in the bloodstream will cause jaundice (the yellow discoloration of the skin) and mucous membranes that can occur with liver disease.

The fat soluble vitamins A, D, E, and K require bile for proper absorption from the intestines. These vitamins are stored in the liver, and are converted to active compounds as the liver maintains normal physiology (homeostasis).

C) Red Blood Cell System

The liver removes old or damaged red blood cells from the circulation and is involved with the storage of iron and the breakdown of hemoglobin. And hence chronic liver disease could cause anemia. The liver (along with the spleen) is a storage organ for blood. If there is a severe blood loss the liver expels this blood into the bloodstream to help make up for the loss.

D) Reticuloendothelial System

Kupffer cells eliminate and degrade the substances that are brought into the liver by the portal vein. Some of these substances are bacteria, toxins, nutrients and chemicals. A diseased liver will not filter these compounds normally, resulting in toxic accumulations of drugs, chemicals, or bacteria. Excess accumulation of bacteria in the blood stream causes septicemia in liver.
E) Vitamins \(^{52-54}\)

Most of vitamins are stored in the liver and perform their functions only when activated by the liver, and are degraded by the liver. These include some of the B vitamins and Vitamin C along with A, D, E and K previously described.

1.6.3 DISEASES OF LIVER

The liver reacts with 8 different types of responses to injury in response to variety of metabolic, toxic, microbial, circulation and neoplastic insults \(^{56-60}\).

A) Degeneration and intracellular accumulation of substances:

Damage from toxic or immunologic insult may cause hepatocytes to take on a swollen and edematous appearance (ballooning degeneration) with irregularly clumped cytoplasm and large clear spaces. Alternatively, retained biliary material may impart a diffuse foamy swollen appearance to the hepatocyte (foamy degeneration). Substances may accumulate in viable hepatocytes, including iron and copper. Accumulation of fat droplets within hepatocytes is known as steatosis and appears in such conditions as alcoholic liver disease and acute fatty liver of pregnancy. A single large droplet that displaces the nucleus, macrovesicular steatosis, may be seen in the alcoholic liver or in the livers of obese or diabetic individuals \(^{56-58}\).

B) Necrosis:

Necrosis is defined as focal death along with degradation of tissue by hydrolytic enzymes liberated by cells. It is invariably accompanied by inflammatory reaction. Necrosis can be caused by various agents such as hypoxia, chemical and physical agents, microbial agents and immunological injury. Two essential changes bring about irreversible cell injury in necrosis – cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by
characteristic cytoplasmic and nuclear changes in necrotic cell. The cytoplasm appears homogeneous and intensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. The nuclear changes include condensation of nuclear chromatin which may either undergo dissolution or fragmentation into many granular clumps.\textsuperscript{56-58}

**Types of necrosis\textsuperscript{56-58}**

**Coagulative necrosis:** This is the most common type of necrosis caused by irreversible focal injury mostly from sudden cessation of blood flow (ischaemia).

**Centrilobular necrosis:** Necrosis frequently exhibits a zonal distribution. The most obvious is necrosis of hepatocytes immediately around the terminal hepatic vein, an injury that is characteristic of ischemic injury and a number of drug and toxic reactions.

**Focal necrosis:** Pure midzonal and periportal necrosis is rare; the latter may be seen in aclampsia. With most other causes of hepatic injury, a variable mixture of hepatocellular death and inflammation is encountered. The hepatocyte necrosis may be limited to scattered cells within hepatic lobules.

**Bridging necrosis:** With more severe inflammatory injury, necrosis of continuous hepatocytes may span adjacent lobules in a portal to portal, portal to central and central to central fashion.

**Submassive necrosis:** Necrosis of entire lobules or most of the liver is usually accompanied by hepatic failure. With disseminated candidal or bacterial infection, macroscopic abscesses may occur.

**Fat necrosis:** The necrosed fat cells have cloudy appearance and are surrounded by an inflammatory reaction. Formation of calcium soaps is identified in the tissue sections as amorphous, granular and basophilic material.
Fibrinoid necrosis: Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall or on the hepatocytes. Local haemorrhages may occur due to rupture of these blood vessels.

Apoptosis: Apoptosis is a coordinated and apparently internally programmed process that mediates the death of cells.

Lytic necrosis: If hepatocytes swell and rupture is called lytic necrosis.

C) Hepatic inflammation

Hepatic inflammation is due to the injury to the liver associated with an influx of acute or chronic inflammatory cells. Sensitized T cells invade the viable hepatic cells to cause liver damage. Inflammation may be restricted to the site of entry of leukocytes in portal tracts or spill over into the parenchyma. When hepatocytes undergo necrosis or apoptosis, scavenger macrophages engulf the dead cells within few hours. Generally clumps of inflammatory cells, foreign bodies, organisms, and a variety of drugs may induce a granulomatous reaction.

D) Regeneration

Regeneration of the cell is observed in the most severe form of liver disease. Proliferation of hepatocytes during regeneration involves mitosis, thickening of the hepatocyte associated with disaggregation of the parenchymal structure to some extent. Complete regeneration is possible when hepatic cell necrosis occurs in the presence of intact connective tissue frame work.

E) Fibrosis

Irreversible hepatic changes, in response to inflammation or by direct toxin action results into fibrosis. Fibrosis is developed initially around the portal tracts or the terminal hepatic vein, or it may be deposited directly within the space of disease.
In the later stage of fibrosis, nodules of regenerating hepatocytes get surrounded by scar tissue which is the site for the formation of cirrhosis\textsuperscript{58-59}.

### F) Jaundice and cholestasis

When bilirubin is elevated in blood and deposited in tissues, it results in jaundice. Jaundice can have a pre-hepatic, hepatic or post-hepatic cause.

**Prehepatic:** In anemia there is an extensive red blood cell destruction that can overload the liver's ability to metabolize bilirubin. This is because anemia can cause liver over load. It usually takes a severe anemia to cause this problem.

**Hepatic:** Icterus can also be caused by impaired excretion of bilirubin in a diseased liver. There is inflammation in the liver and biliary system and the swelling is known as cholangiohepatitis. This impairs the liver’s ability to excrete bilirubin in the digestive system. The bilirubin builds up and eventually spills over into the bloodstream, causing the yellow discoloration.

**Posthepatic:** Icterus can also cause by obstruction of bilirubin flow out of the liver, which is a more extreme version of impaired excretion. These animals will have light colored feces because no bile pigment is being excreted into the digestive system to give stool its dark color. Bilirubin that is retained in the liver is toxic and will add to the liver problem that is already present. Whereas failure in the biliary secretion results into cholestasis. Cholestasis is accompanied by the accumulation of substances in blood normally exerted in bile which includes bilirubin, cholesterol, etc\textsuperscript{56-60}.

### G) Ascites

When excess fluid is accumulated within the peritoneal cavity it is termed as ascites. Ascites can be clinically detectable when at least 500 ml of fluid is accumulated\textsuperscript{59-60}.  

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1.7 HYPOTHESIS

There are hardly any proven remedies for the prevalent liver disorders among people. No drug has been developed in the modern system of medicine which many stimulates the liver function, protect it from damage or help in the regeneration of hepatic cells. The only drugs, which are available for treatment of liver disorders, are corticosteroids and immuno-suppressive agents but their use is accompanied by serious side effects. There is an ever-increasing need for an agent, which could protect the liver against damages. Though numbers of such plants viz. Acacia catechu, Picrorrhiza kurrooa, Swertia chirata, Piper longum etc. have been previously investigated for their hepatoprotective nature and some Ayurvedic drug i.e. Kumari asympt, Tamra bhasma have also shown the same property. But information is still lacking with regards to the precise action61.

Since worldwide efforts to find a safe hepatoprotective agent are in progress, hence the present study was carried out with Coccinia indica, Sida cordata and Scoparia dulcis leaf extracts. The study was designed to investigate the effect of different extracts of all three plants in CCl₄ induced hepatotoxicity in rats. The study also includes in vitro antioxidant parameters, since antioxidant also play a critical role in free radical scavenging and protection against various elements.

In the present study we are developing a polyherbal formulation comprising of extracts of Coccinia indica, Sida cordata and Scoparia dulcis. When many herbs are mixed together we can expect a better activity than the individual herb. This may be due to synergism as well as different mode of action of individual herb. In the present study we are trying to develop a polyherbal formulation comprising of herbs of different mode of action. Coccinia indica is known for its antioxidant activity and Scoparia dulcis is very good immunomodulator as well as antioxidant herb.
*Sida cordata* is indigenous medicinal plant and used by the traditional herbal practitioners of Chhattisgarh for curing of liver alignment and other diseases. When we mix these herbs of different mode of action, we can get a better hepatoprotective preparation, whose activity may be comparable to the existing polyherbal formulation like Liv. 52 etc.

So our hypothesis is-

"A polyherbal formulation containing different extracts of *Coccinia indica*, *Sida cordata* and *Scoparia dulcis* will be having potent hepatoprotective activity against various hepatotoxins like CCl₄, etc. which can be comparable to that of well-known liver tonics available in the market e.g Liv. 52 etc".