1. INTRODUCTION:

1.1: INTRODUCTION

For thousands of years mankind has known about the importance and benefit of drugs from nature. The crude drugs which are obtained from the plants or animal and some of the inorganic salts used by old or ancient civilizations. The medical plants have been estimated at very highly, for example – Indian Traditional System where the Ayurveda has been reported variety of medicines from plants during since 1000 BC. The as per the prescriptions or reference which are reported in Chinese medicine based on natural products dated back about to 500 BC and some of the classical Chinese formulation which are down in the years between 25 and 220 BC and these are used still [32].

The Nature has been always make a Golden Mark to situation the outstanding phenomenon of evolution of plants and animals. The plants are plays role of indispensable to man for his life. There are three important necessities which are required of for life i.e shelter, food and clothing and a basic important of other useful products which are supplied by the plant kingdom. Nature are provided a definite storagr-house of remedies which were to treated all type of diseases of human. About thousand of year before the natural drugs has been utilized for the treatmaint of several diseases and the drugs which are obtained or find out from nature. So, now a day we are used natural drug and ensuring regarding health care [57].

Nature is beauty in terms of health care is appreciated by developing various systems of traditional system. The Ethnomedicine may be defined broadly as the used of plants by human as medicine, but this is use could be called ethnobotanic medicine. So, they are given the importance as well as which are focus on the traditional drugs in the Indian system of medicine and care system (for example - Ayurveda, Homeopathy, Yoga and Unani) for the solve the problem regarding health care. The people having awareness about the plant drug and herbal remedies obtained from plants, animals and minereal origin and represent their part of the global market [63]. The pharmaceutical companies are going to expend the huge amout on the research related to the crude drugs or plants materials for there potential medicinal uses. Because today near about 80 % people were going to use herbal product. Therefore, the increases the demand for herbal products. India is one of the leading biodiversity point in the world and also one of the oldet, richest and the use of plant drug [91].
The disease is the basic problem which is faced by humans too since prehistoric times. Nature has along with the disease created their cure in the form of vegetables, minerals and animals. The relationship between man and plants have been close throughout the development of human culture. The basic problem of mankind therefore understanding of human disease there has been continued interest in the drugs from the plant kingdom.

As per the literature survey or report of great physician of India know as Harappa and Mohenjodaro has been reported most of usfull or important herbal drug and also reported there significant evidence were reported. Even people saw that Ayurveda is the very old system of “Indian indigenous medicine system” as well as its important part of the Indus Civilisation. Rigveda is the very oldest documented reported about plants and herbal medicines.

In India the use of Medicinal herbs which are very old as 5000 BC. Hence known as the India traditions as well as folk traditional system. According to the literature of Sidha in which reported the several medicinal value of the plants. About 6000 years old before number of crude drug were used for the treatment of several diseases the evidence has been reported. It is estimated that near about 90,000 species are available and which are usefull for the several types of Indian system of medicine.

The importents knowledge about the plants and plant produced products is rather detailed and sophisticated information has been reported in book which is know as Dravya Guna Shashtra. About 26,000 drug formulation and crude drugs have been studied and about 50,000 formulations were reported. The Vaidyas, poems contain having rich sourc of material on the Herbal role of that time [68].

**Indian Traditional System of Medicine:**

As per the Indian System of Medicine, in which most of the drugs come down in several foms of them which strand found easily. In encient or old time used the crude drug formulation for the treatment of diseases and the formulation making by traditional practicener, also known as Vaidyas or Bhishaks which gives treatments for various diseases by using the plant drugs. Vaidyas or Bhishaks originally belonging to a class of people known as the Ambashtas. Mostly the drugs which are of tribal origin and which are obtained from herbs and plant origin drugs collected from forests as well mountains and herbs are sold in villages. Then third group
comprises temple priests, especially for the Vaikhanasa persuasion, also required to function as physicians.

Ayurveda consider human being existing the five elements called as Prakruti which included vatta, pita and cough. When the imbalance of Thri-Doshas, then which are produced the disease and the restoration of balance which are helps the removed the disease. The objective of the treatment is not only to treat the disease but also to wash out the root cause of the diseases. So, that it may not take place in future. The main aim of the remedies are also to improve the validity and to increases the strength of immune system. The identity of drug plant was based on the doctrine of signatures, viz, “Pashanbhed” meaning thereby the plant. It is also indicative of dissolving or splitting of the stones in the bladder or kidney. The doctrine of signatures was not often recorded, but the knowledge was handed down from “guru” to disciple or father to son where the Ayurvedic profession was confined to the family.

These are traditional system of medicine which compose three system and these are Ayurveda, Siddha and Unani practiced by Hakkims, Vaidyas and Siddhs respectively. These all medicines were that come under Ayurveda, Siddha and Unani system of treatment are known as Indian System of Medicines. The Drug and Cosmetic Act stated that "Ayurvedic, Siddha and Unani system in which involved all medicines, which are used for the determination of the internal or external diseases, and treatments. A detailed study of ayurveda can suggest the activities, which can be tested by modern technique. The Natural products are quality and the standard of efficiency and safety as applicable to any modern drug should be applied and if found acceptable, the only they become real ayurvedic remedies. In these times of drug induce disease and drug price causing economic catastrophies in families and to the country, it is necessary to take resource to ayurvedic not merely herbal remedies for wider benefit of ailing humanity and living being the world [97,93].

Research and Development of Plants

Nature produced always important drug source and surprising opportunities afforded by pharmacognosy, phytochemistry and natural chemistry these three departments doing continuesally research and produced new compound which are usfull for the treatment of various diseases.

Natural products, included plants, animals as well as minerals drugs and which are utilized for the treatment diseases on human. Even the used of crude medicine are existence
before cristian of civilization. The continuously efforts of researcher and scientists were developed new modern medicine over the years by scientific. Therefore, according the to the work report shown the researcher has development of traditional medicine which are used. The history of medicine consists of many laughable therapies. As per the oldest knowledge the natural medicine one important source of as future medicine which are used as a therapeutics [72].

In terms of modern research to development of plant drug should be required for the multi-disciplinary approach. Now a adys drug designe studies of Ethnomedicine information, and as per research report of biological screening, have been never confirm that plants are a mediater of chemical agents which are having therapeutic importance. Then, where has been something missed is collection of data gathered in the trial as well as clinical trial. The Unidos technical assistance programme observed that if a successful plant was evaluated. Hence, FAO was increases the cultivation or plantation of plant and also implemented clinical trial assessment of the crude drug be conducted according to Word Health Organisation participation. The number of new programme was introduced for achiving the goal of research related plant drug [19].

The importance of plants using as a source of therapeutic agents are-

a) After separate active constituents used directly.
   For Example: taxol, digoxin, digitoxin, vinblastine, morphine, vincristine.

b) The uses of agents as pharmacologic tools.
   e.g. yohimbine, lysergic acid diethylamide.

c) To utilized the part for a herbal remedy.

**Drug Discovery by Ethnobotanical:**

In the pharmaceuticals industries isolation of vincristine from the Vinca for the drug discovery has been proved successfully. According to the ethnopharmacological research has been discovered new pharmaceutical compounds for treatment of HIV and anti-inflammatory compounds.

As per the data of ethnobotanical having the crucial three types of drug discovery are follows -

1) The natural plant products which are unmodified where the ethnomedical are suggested that which are clinical efficiency. For example – *Digitalis purpura*
2) The natural products of the crude drug which are having the therapeutic efficiency. For example- vincristine.

3) The Modified natural of substance which are produced from synthesis of drug. For example - aspirin [26].

Fig.1.1: The schematic diagram of traditional medicine.

**PERSPECTIVES FOR INDIAN HERBAL MEDICINES :**

The mostly developing countries such as India having strong traditional knowledge and having the potential to develop new compound. So, that is reason behind increasing of herbal company in India. The Herbal products are a very strong traditional medicine and which are useful drug used as a safety and having the significant value but they have lack of knowledge about research base and hence they have after first class value and the modern medicines have a strong result as well as on the basis of experimental which are the usefull but these type of medicine having side effects. Therefore, about 85 % people in the whole world trust on the alternative systems of medicine, and which gives the effective result and these are the most significantly used, because of following reasons,

- Claimed to be safe
- To compared to allopathic drugs
The herbal remedies not having ADR.

In development of drug and pharmacological research, the medicinal plant or the herbal drug are most widely and commonly used for screening purposes. These medicinal plant constituents are act as a therapeutic agents and also used as the synthesis of new drugs and new compound. These synthesized new compounds shows pharmacological activity most significantly and most effectively. A sufficient amount which are spend to research on herbal formulation, therapeutic activity and investigation of phytochemistry which have been carried on medicinal plant constituents. In this number of molecules have came out from Ayurvedic research base, included alkaloids used for the treatment of hypertension, hypolipidemia. Mucuna pruriens is used for the treatment and prevention of Parkinsonism. The piperidines derived from plants is important for enhancing bioavailability. Baccosides are important for mental retention, and in treatment of liver diseases embrica officinale is used. , phyllanthins as antivirals, curcumines used for reduced for muscle pain, many other steroidal and their glycosides have been used for treatment of immunomodulators.

Most of the plants used as a medicine in the Indian Systems to find novel compound for treatment of different diseases and evaluated the drug is most important. For the determination of herbal drugs has some parameters like phytochemical investigation, to evaluated quality and standardization of the raw materials as well as finished products, pharmacokinetics and herbal pharmacovigilance, herbal formulation, shows the efficacy and benefit of natural resources. They not only help to prove the medicinal effect and benefit of these alternative systems but they also give more and more benefits of the drugs from natural sources \[64\]. The appropriate methods used for the manufacturing and quality control of herbal drugs, and in research and development of herbal drugs in Ayurveda and in ayurveda therapeutically the drug is mostly investigated and evaluated by using the additional scientifically used methods shows the most effect and benefit of the products to the human beings.

**CURRENT STATUS OF STANDARDIZATION**

WHO has particularly on the need to confirm the quality control of herbs and herbal formulations by using modern techniques. Number of Pharmacopoeias Pharmacopoeia, Ayurvedic Formulation, United States Pharmacopoeia, Herbal Compendium, and German Commission etc. refered monographs for herbs to maintain their quality. In Ayurvedic herbal drugs, ayurvedic pharmacopoeia of India which is most recommends the parameters commonly
which have a basic quality. British Pharmacopoeia which contains about 235 monographs and quality control tests, Chinese Herbal Pharmacopoeia contain about 1757 monographs of substances and articles. German Commission also reported about 335 monographs for drug used in German folk medicine.

**STANDARDIZATION BY MARKER COMPOUND**

The best tool developed for standardization is by chromatography. It describes botanical identity and chemical stability of herb. One of such technique is marker compound testing and finger print about 1500 analysis.

Some of the Secondary metabolites present in herb are considered as marker compounds:

<table>
<thead>
<tr>
<th>Name of Herb</th>
<th>Marker Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrographis paniculata</td>
<td>Andrographolides</td>
</tr>
<tr>
<td>Boerhaavia diffusa</td>
<td>Punarnovine</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Curcuminoides</td>
</tr>
<tr>
<td>Eugenia caryophyllata</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Glycyrihiza glabra</td>
<td>Glycyrrhizine</td>
</tr>
<tr>
<td>Withani somnifera</td>
<td>Withanolides</td>
</tr>
<tr>
<td>Tinospora cardifolia</td>
<td>Giloin</td>
</tr>
<tr>
<td>Piper longum</td>
<td>Piperine</td>
</tr>
<tr>
<td>Ocimum basilicum</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Gingerol</td>
</tr>
</tbody>
</table>

Several chromatographic methods are used to estimation of the standared components which are obtained from herbs. Even for interpretation components there are different methods like HPTLC, HPLC etc. [26].
<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Category</th>
<th>Plant Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ajmalicine</td>
<td>Circulatory disorder</td>
<td><em>Rauwolfia serpentina</em> (L.) Benth ex. Kurz.</td>
</tr>
<tr>
<td>2</td>
<td>Aesculetin</td>
<td>Antidysentry</td>
<td><em>Fraxinus rhynchophylla</em></td>
</tr>
<tr>
<td>3</td>
<td>Arecoline</td>
<td>Anthelmintic</td>
<td><em>Areca catechu</em> L.</td>
</tr>
<tr>
<td>4</td>
<td>Atropine</td>
<td>Anticholinergic</td>
<td><em>Atropa belladonna</em> L.</td>
</tr>
<tr>
<td>5</td>
<td>Bromelain</td>
<td>Anti-inflammatory;</td>
<td><em>Ananas comosus</em> (L.) Merrill.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>proteolytic agent</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Berberine</td>
<td>Bacillary dysentery</td>
<td><em>Berberies vulgaris</em></td>
</tr>
<tr>
<td>7</td>
<td>Caffeine</td>
<td>CNS stimulant</td>
<td><em>Camellia sinensis</em> (L.)Kuntze.</td>
</tr>
<tr>
<td>8</td>
<td>Curcumin</td>
<td>Choleretic</td>
<td><em>Curcuma longa</em> L</td>
</tr>
<tr>
<td>9</td>
<td>Cocaine</td>
<td>Local anaesthetic</td>
<td><em>Erythroxylium coca</em> Lam K.</td>
</tr>
<tr>
<td>10</td>
<td>Colchicine</td>
<td>Antitumour agent</td>
<td><em>Colchicum autumnale</em></td>
</tr>
<tr>
<td>11</td>
<td>Digoxin</td>
<td>Cardiotonic</td>
<td><em>Digitalis lanata</em></td>
</tr>
<tr>
<td>12</td>
<td>Emetine</td>
<td>Amoebicide</td>
<td><em>Cephaelis ipecacuanha</em></td>
</tr>
<tr>
<td>13</td>
<td>Glycyrrhizin</td>
<td>Sweetener</td>
<td><em>Glycyrrhiza glabra</em> L</td>
</tr>
<tr>
<td>14</td>
<td>Hyoscamine</td>
<td>Anticholinergic</td>
<td><em>Hyoscamus niger</em> L</td>
</tr>
<tr>
<td>15</td>
<td>Morphine</td>
<td>Analgesic</td>
<td><em>Papaver somniferum</em> L</td>
</tr>
<tr>
<td>16</td>
<td>Quinine</td>
<td>Antimalarial</td>
<td><em>Cinchona ledgeriana</em></td>
</tr>
</tbody>
</table>

Table 1.1: List of Drugs derived from plants.

Some new drugs are administered by this route, which specially are those routes and drugs which are used to treat infectious diseases, heart disease like blood pressure, arrhythmia, cancers, gastric ulcers, infestations etc. there are number of drugs which are synthesized and manufactured by using chemically derived compounds act as a dye-stuffs. The drugs like penicillin, isoniazid, some antipsychotics, antihistamines are occurred from serendipity which arises from scientists and physicians observations by visualizing sharply emergence of the modern pharma. Industry produced or developed new single compound which having highly activity for a wide variety of disorder. The drugs are produced in many cases developed on
nature, namely a new range of anaesthetics from cocaine. It avoided because the drug having
dangerous effects on BP; as compared to quinine chloroquine is much less toxic. It is very
necessary to produce additional medicinal gardens continuously in every country and all over the
world. The crucial point are to check the efficacy of drugs these drugs are usually because complex
mixture was not evaluated the analysed only crude. Therefore, the plant medicines became the
origin from the plants, animals and mineral. Natural compound continued to reflect poor quality
control both for raw materials and finished product. The combinatorial chemistry rapidly and
recently developing the number of new and different chemical compounds, but these compounds
are not possible to produced from the natural sources. So, that type of compounds lead to the
discovery of traditional drug that will exactly increased the area of efficiency of chemical
research.

Lead finding:

Nature has provided almost extremely great source for the designing and development of
the new drug. In the world there are existence of a number of species and organisms, but their
collection is very difficult and not be collected easily. The most important things that the plants
has part relatively small group which are about 2,60,000 species, and out of that which
approximately 7% of species has been studied for biologically screening, and near about 17% species has been studied constituents. A number of metabolites like primary, secondary are
produced by the above mentioned specific organisms with its suitable environment. The number
of secondary metabolites are available presently about 140,000.

Every year near about 4000 new structures have been reported. The vocabulary or list of
natural products is a collection or arrangement of total chemical compounds and this vocabulary
includes nearby 140,000 compound entries. The existence mostly terpenoids are presently, and
the second largest group included that is alkaloids.

Now presently extremely large related structural diversity, nature utilized only a some
basically metabolic products or catabolic compounds like phenyl propanoid (C₉), The acetate
(C₂), isoprenoid (C₅) and units etc. For nature has opened for the chemodiversity, in recent days
and recent years the condition and situation of the new techniques and new methods of
identification for the development and introduction has been changed. By using various methods
the several number of sampling compounds and molecular targets are checked and evaluated for
their therapeutic activity. The number of new synthesized compounds are not mostly produced by the chemists as comparing to those with the natural products.

Therefore, there is always shows the structural variety between synthetic compounds which are produced from synthetic chemistry and natural product, they never match to each other. Thus a new synthesized active compound can not be designed in synthetic laboratory. Thus, it is possible produced and developing drugs from natural compounds. The drugs which are originated from plants and animal used to evaluate and identify the extracts which are obtained in large numbers and are very useful and proper pharmacological evaluation. In chromatographic methods, the Thin Layer Chromatography technique was very important for the natural products and also useful in the to check the activity of medicinal compounds which are separated from extracts of the medicinal plants by the chromatographic methods. So these methods are powerful techniques which are plays important role for the investigation of chemical constituents and separation of medicinally active drugs or compounds from the extracts of medicinal plants and most effective for the new drug developments. For the development and search of newly active biological compounds, obtained from plants or extracts of the plants, there are several spectrometric methods like Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) which are commonly used. The cell culture extracts form plant these are another option for screening or observed biological activity. For the development of new compounds, there are several plant cell cultures are used, and by using culture techniques the produced compounds gives the significant and effective activity. A number of different varieties of approaches used to finding lead which from the study of systems of traditional medicines. Now a days the increases of awareness of in developed countries that their cultural and move forwarded and like the hearbal medicine, studied of traditional medicine are receiving more attention. Hence, it is estimated that about 80% of the world population belive s on traditional medicines in primary teratment. The active constituents are responsible for activity in traditional medicines [95].

There is an urgent need to confirmation of identity of novel drug, active chemicals as leads for effective drug development. It has been estimated that near about 15 % of plants origin drugs have been investigated by systematically for the present of bioactive compounds. While the potential of marine environment has barely been tapped [17].

**Molecular Pharmacognosy:**
The subject of Pharmacognosy stated that study of crude drug which are obtained from plants, animals and mineral origin. The plants which show toxic as well as harmful or curing properties were discovered by humans in their search for food [70]. The knowledge of Ayurvedic which is play important role for the separation of drugs in modern science and characterized as well as standardize of the active chemical constituents from natural drug.

“Pharmacognosy is a molecular science that explores naturally occurring structure-activity relationships with a drug potential” The term “molecular” here has a broader meaning than in “Molecular Biology” and other sciences which have included molecular as a part of their name to imply a focus on enzymes and genes. In the definition of molecular Pharmacognosy “molecular” simply means that it is sciences which focus on molecules and involves isolation and determination of the structure of pharmacologically active molecules as well as the study of their biosynthesis, including the enzymes and genes which are involved. Also the term “structure-activity relationships” is used here in a broader sense than is commonly the case in medicinal chemistry. The definition also restricts the research activity to molecules which could be used as drug, thereby stressing that Molecular Pharmacognosy is a pharmaceutical research subject also suggest the following explanatory model [99].

The starting point is an organism (Upper left corner) which displays some kind of biological activity (upper right corner). Bioassay direct separation from the complex biomass will lead to the identification of a chemical structure (central) which can be correlated to the observed biological activity.

The chemical structure can then be used for development of a drug (lower left corner) or as a model, precursor or tool in the drug research (lower right corner) which are represent Fig. 1.2.
Fig 1.2: Explanatory model for molecular Pharmacognosy

MODERN DRUGS FROM AYURVEDA
In Indian culture and civilization the Ayurveda is the Indian traditional system of the medicine. As per earliest-literature knowledge of Ayurveda is included in the *Atharvaveda and Rigveda*. The *Atharvaveda* listed eight divisions of Ayurveda such as paediatrics, internal medicine, surgery of head and neck, surgery, toxicology, ophthalmology, psychiatry, science of fertility and science of rejuvenation. The great vaidya Susruta work as Surgen between 700 BC, in Banara University, he has developed the operative techniques of plastic surgery, and written book i.e *Sushruta Samhita*, mentioned a highly developed surgery. Th supplemented the *Atreya Samhita* revised by great physician Caraka reported the drug in his book, Charaka reported near about 600 drug which are obtained from animals, plants and mineral and about 650 in the *Sushruta Samhita*.

Indian Ayurvedic system several as given birth to number of drugs. The importance of plants as a source of useful antihypertensive drugs was supported by the isolation of reserpine from *Rauwolfia serpentina* by Muller et al in 1952. Veratrum alkaloids were other antihypertensive agents from plant sources. Recently there are a number of medicinal active constituents obtained from plants which have shows the activity against thrombocytes. notable among these are *Curcuma longa*, *Coleus forskohli*, *Zingiber officinale*, *Allium sativum*, *Commiphora mukal and Cuminum cyminum*, etc. Many of these are also mentioned to be useful in cardiovascular ailments in classical text books of ancient medicine.

**CURRENT SCENARIO OF HERBAL DRUGS:**

Curantaly day by day increasing the utilized of herbal medicines widely distributed in development and production in over all the world. the countries which are situated in Europe have near about 80 % people used plant origine drugs. According the Export–Import egencies reports shown that the plant-derived and obtained fro plant products are about $60 billion of US dolor where India would reach near about 3 trillion US$ by the end of 2015.

World Health Organization (WHO) were shows About 25,000 or nearby organisms obtained from extracts of medicinally active plants. According to literature reporte the medicinal drugs used individually nine thousand two hundred for thirty three thousand species monocots, lichens, bryophytes, dicots, gymnosperms, from that nearby about 30 % of plants suggested which are found on the earth. These are used as a ethno-medicinally [28].

According the data shown that the India has been identified as one of the medicinal drug exporting country in the world. India has medicinal plants diversity determined that is near
about 90% of the types of forests are available in India and about that 3.00-4.00% are considered with 10% of global biodiversity. According to a report, about 10% of the known medicinal plants and about that most of the plants widely spread and distributed for the purpose of medicinal and therapeutic activity throughout the India.

1.2: INTRODUCTION TO HEPATOTOXICITY

![Diagram of Liver](image)

**Fig 1.3: Diagram of Liver**

The liver is a wedge shaped organ which is situated below the diaphragm and upper part of the abdomen. The major part of the liver is the right lobe and left lobe which are situated with its apex. In healthy human contain average weight of liver is 1.5 kg, the liver has two inferior convex and superior convex surfaces with two borders such as anterior posterior borders. The liver is divided into two parts known as lobes i.e right lobe which is large and left lobe by a falciform ligament. The upper surface of liver contains left lobe, right lobe, triangular ligaments, inferior vena cava etc. the liver which is made up of lobules these lobules contains the liver cells. The connective tissue which is present in the lobules and that consists of blood
vessels and these blood vessels passes the blood to the liver and blood circulation of liver occurs. The hepatic artery and hepatic veins are present in liver lobules. Blood is passes to the liver through spleen, stomach, intestine, pancrease.

The liver is considered as the lever of all body mechanisms because of, in liver all metabolic process are occurs. Liver synthesizes, stores, excretes, generates, metabolites, secretes, detoxicates, protects to the human body. Liver is considered as the key of health for the humans as well as animals. Carbohydrate metabolism, protein metabolism, fat metabolism are occurs in the liver. The glucose is obtained from all mono, di, and polysaccharides in the liver. It plays important role in the blood coagulation process. It is also useful in the synthesis of vitamins. For vitamia A and D it is the store house. In foetal life erythrocytes are synthesized in the liver. The liver also synthesizes albumin, globulin, fibrinogen, and plasm proteins.

For all the metabolic processes, The Liver act as a very important role for both intra and extra cellular compounds. If there is regularly involvement of those compounds, they are produces toxic injuries which are caused by several hepatotoxic agents and any damages to hepatic cells disturb of human body metabolism. Now a days mostly people are developed or find out the natural remedies which are benefit or important for treatment of the hepatic disorders which are induced or caused from various toxic agent such as parecetamol, alcohol and hepatic virus etc. [22].

There are no remedies for treatment of liver disorders, but which are commonly present in the human beings. The Scientists, research scholar and Industrialists looking possitivaly about plants drug and they are seriously on various prospective plant remedies for diseases of liver disorder management. The World Scientific Community which is concentrated on an herbal plant drug during the decade 70s and important drugs is Vinca Rosa. After that in 80s the become importance was focused on Panax ginseng. Now, according the literature the Neem plant are very importants which is having various potential for the treatment of diseases. So, Neem tree has indicated that it become centre for research in 90s centuary. The ICMR, New Delhi, according the revived research on traditional sysyrm of medicine, had adopted liver diseases as some among six thrust areas and for more than one study. Identification and estimation of active constituents from Picrorhiza kurroa, Bhoomyamalaki (Phyllanthus niruri) have shown significant activity protection against the liver disorder like as a Jaundice. In urban areas of India, the Hepatitis disease is most commonaly found as a major health problem as viral diseases.
like hepatitis. Therefore, the research or investigation under Research centres such as ICMR. As per the study of the Indian Society of Gastroenterology, the *Glycyrrhiza glabra* is important plant drug which is killed the viruses which are present in liver cells.

The diseases of liver may be - chronic hepatitis or acute hepatitis also known as inflammatory liver diseases, the hepatosis called as non inflammatory liver diseases. The hepatic damage of Liver indicated the determination of enzymes as an index of sub-clinical parameters. Serum alkaline phosphatase (ALP), Serum glutamic pyruvic transaminase (SGPT), Serum bilirubin and Serum glutamicoacetic pyruvic transaminase (SGOT), were indicated as an injury of hepatic cells in the liver [46].

1.2.1: ANATOMY AND HISTOLOGY: [92]

**Anatomy:**

The liver is a larger gland in the body which is situated in the right upper part of the abdomen or stomach. It is a solid gland. The Liver is having slightly reddish brown colour, which is in soft consistency and it is very friable. It average weight is about 1.5 kg in healthy humans. The liver occupies the whole of the right hypochondrium.

![Fig 1.4: Anatomy of liver](image-url)
**Histology of the liver:**

The liver is divided into two parts by a ligament and each part is considered as a lobethe right lobe is slightly greater than left lobe. The lobule made from functional units of the lobes of the liver. The structure of lobule has six sides which are arranged in irregular shape, interconnected branching plates with a central vein. The sinusides are the large endothelial spaces of liver. With the help of sinusides the liver passes the blood. The stellate reticuloendothelial cells, which destroy, worn out white blood cells and erythrosites, microorganism and impurities, toxic materials in the venous blood drain by the gastrointestinal tract.

**The Blood circulation in the liver:**

In the liver, circulation of blood, the both arteries and vein present in liver from that blood passes to the to the liver and these are hepatic veins and hepatic arteries. The oxygenated blood carried by hepatic arteries and deoxygenated blood is carried by hepatic vein which contains drugs and possibly microbes, absorbed neutrients and toxins from GIT. The hepatic artery and the hepatic vein carry or pass blood into sinusoids of liver. In sinusides of liver, the hepatocytic cell carries oxygen, nutrients like proteins, vitamins, etc. and several toxic compounds.

**The Functions of Liver:**

**Carbohydrate metabolism:**

The liver plays important role in the synthesis of glucose from all the mono, di, and polysaccharides. When level of blood sugar is reaches low, then, the liver may break down glycogen to glucose and release glucose into the bloodstream. When the concentration of glucose in blood is highest, the liver undergoes the process of glycogenesis in which glycogen is synthesized from glucose and also there is a formation of triglycerides and these are the main storage components of the liver. In Liver glucose is also synthesized from non-carbohydrate sources like amino acids, gyerol and lactic acid etc. and this process is known as gluconeogenesis.

**Lipid Metabolism:**

The Hepatocytes store several fatty acids like triglycerides, and degrade fatty acids to generate ATP. For the production of bile salt several synthesized cholesterol is used and this cholesterol is synthesized from liver.

**Protein Metabolism:**
In liver the catabolism of amino acid is occurs in which ammonia is removed from amino group of amino acid by the process deamination or trasamination, but synthesized ammonia is more toxic. Hepatocytes determinate amino acids so that the a to the body and it get converted to the non toxic compound urea by the liver. Some times the amino acids can be used for the formation of carbohydrates like glucose and there is a formation of richest energy molecule ATP.

**Prevation of toxicity:**

Mainly the liver prevents toxicity of substances like alcohol or liver excreted such compounds like penicillin, sulfonamides, erythromycin etc. into bile.

**Storage:**

The liver is the store house of vitamins like retinol, calciferol, cynocobalamine, tocoferol etc and number of substenances such as glycogen, minerals, and these are realesed from liver.

1.2.2: STUDY OF HEPATOTOXICITY: [56]

**Damage liver cell changes**

Hydropic change of liver cell is a more described word which applies to the liver cell with slightly faintly, water containing cytoplasm and a nucleus. Most of the variety of conditions develops the colouring or staining of cytoplasm which is relatively less. The eosinophils are increased in the blood may be find with compound related hydroplasia of the endoplasmic reticulum (smooth) i.e ribosomes are not attached to the E.R. Active regeneeration of liver cells after liver damage occurs in hepatitis occurs due to viruses or recovery phases of fatty liver created a widespread damaged liver cells changes as well as a cobblestone typed of the liver cords. Due to the drug induced hepatotoxicity or due to the alcohol induced liver damaged shown are the damage and indicated the viral hepatitis and changes of the or recovered the hepatoprotective drug.

**Histopathological basis:**

Hepatocellular fat accumulation may be either large cytoplasmic body composed of minute fine foamy vesicles of fat within a hepatocyte. The liver of Fatty which occurs because of has some reason these are

- The increase imidiataly in movment of fat from the periphery in the liver,
- Lack of protein is essential for hepatocellular fat release,
- Increase hepatocellular fat formation by metabolic changes and
Decrease hepatocellular fat degradation. Fatty liver is common in alcohol ingestion, parental nutrition, tuberculosis, starvation, and certain drugs.

**Hepatocellular Necrosis:**

The hepatocellular necrosis has classified in various types, these are introducing at particular part (Periportal, pervenular and so on), mechanism (lytic, coagulative), amount (submassive versus focal), cellular type (Lymphocytotoxic versus hyline necrosis) and various pattern are composition having different causes diseases. When the acute hepatic injury the common pattern shown like zonal necrosis. The actually coagulation of liver necrosis are having certain typical of acetaminophen injury and can be discussed by various oxygenation and also having the activity of enzyme drug metabolizing. The primary liver necrosis is not generally and did not indicated coma and convulsions during pregnancy. The Midzonal damage is having for yellow fever.

The damaged liver or necrosis of hepatocellular are the lytic type, joined with the small activity that are generally dead hepatocytes are rarely noted; such type of liver necrosis are common shown in widely spread viral hepatitis in the liver, the hepatotoxic reaction due to the given of alcoholic. The composition of necrosis in the liver is having different conditions. This typed parameter are shown by coloring hepatocytes that removal of some staining of the cytoplasm due the inflammatory action the cells being shrunken and the cells are slowly disappear because of the inflammatory action..

**Regeneration:**

The liver cells are having a important capacity for regeneration of cell and it has been recovery the submassive hepatic cell which are indicated death of tissues by enzyme degradation. In liver of adult rat, the liver cell is having capacity of turnover of one mitosis per year and occure weight doubles within 48 hours. The liver cells weights are normally recovered within 3 to 6 days. The human livers have capacity regeneration also having increases and also regeneration in interstitial inflammation of liver. When major hepatic (an agent that acts on the functions of liver) resection completes for tumor or malignancy, the normal volume of cells are regenerated within 3 to 6 months and after surgery the liver function has nirmaly work.

**The liver Fibrosis:**
The Hepatic formation of fibrous tissues (fibrosis) are plays crucial role when the chronic liver cells disease and increases to the cirrhosis and liver function are changes which are produced various clinical manifestation of fatal liver disease.

Types of fibrosis: Simple hepatocellular necrosis does not result in collagen formation, but in severe hepatic necrosis a collapsed stroma may form a framework for other retention of collagen. The continuous or regular necrosis occurs with fibrosis and the most striking fibrosis within the lobule is noted in chronic alcoholic disorders of liver. The formation of fibrous tissues occurs even with a very less response of inflammation. Another same pattern of formation of fibrous tissue occurs in the portal areas in chronic active hepatitis which tends to be more confirmed to the portal tracts and does not extend into the lobule as much as portal fibrosis does in alcoholic.

Liver Cirrhosis:

The presentally liver cirrhosis stated that cirrhosis are needed the term to be applied of the liver which are diffuse liver fibrosis (that is entire liver and not focal) and which contains regenerative of nodules which are lobes of hepatocytes decreases the normal blood flow due to the lack of terminal hepatic cells. The cirrhosis are divided in different class as micro or small nodular having the three millimeters in diameter and out of that some are less than 1.5 millimeter in diameter.

The macronodular cirrhosis which are applied when nodules are greater than three millimeters in diameter and these are available in two different forms. The nodules divided by thin septa that are incompletely and have linking an entrance tract.

1.2.3: ENZYME THAT DETECT HEPATOCELLULAR NECROSIS: [85]

Nearby thousands of enzymes are present in liver, but some of them are in very low concentration which are present in serum. The enzymes which are present in serum have no more functions or also having other function and behave like other proteins. The hepatocellular necrosis are distributed in plasma as well as intestinal fluid and they are have specific character are no appearance, they generally measures in days. When the increased rate of liver cells damaged in serum then increases the enzyme activity in serum. The Serum enzymes tests can be grouped into two categories these are –

1) If the enzymes whose increases in damage to hepatocytes and
2) If the enzymes which are elevation in reflects of cholestasis in serum.

**The Aminotransferases**

The enzyme transaminases like serum aminotransferase are more sensitive indicator of liver necrosis, and useful in identifying acute hepatatic disorders such as hepatitis. Alanine aminotransferases (ALT), serum glutamic-pyruvic transaminases (SGPT)) and Asparate aminotransferases (AST), serum glutamic-oxaloacetic transaminases (SGOT)), these are the important biochemical parameters which are measured activities in serum of liver.

All enzymes are protein in nature so the enzymes derived from the amino acids such as aspartic acid, alanine etc. catalyze the reactions in krebs cycle, glycolysis. The ketoglutarate forms the pyruvate and oxaloacetic acid. The number of methods used for measurement of Alanine aminotransferases and Aspartate aminotransferases activity or mechanism in serum, in that the most significant and effective method which are helps to the synthesis of oxaloacetate and pyruvate. The enzymatic reduction to lactic acid and malic acid. Reduced form of the coenzyme such as nicotinamide adenine dinucleotide (NADH) which is derived from vitamin B, the cofactor in this reduction NADH get aerobically converted to nicotinamide adenine dehydrogenase (NAD). Which the light is absorbed at 340 nanometer and that events can occurs as follows.

By the Spectrophotometricaly the low absorptivity of 340 nm, and the both the aminotransferases enzymes are generally present in serum at low concentrations, and they are having less than 30 to 40 IU/L.

The Asparate aminotransferases which is shown in the liver, as muscles like skeletal, cardiac, the brain, kidneys, the lungs, the pancreas, which are in decreasing order of the concentration, where alanine amino transferase is present at largest concentration in the liver. If the ALT and AST serum values are increase then damage of tissue they changed the permeability of cell membrane. Hence, the ALT and AST are allowed to the leak into serum.

Aminotransferases typically are elevated in all liver disorders. This includes the cirrhosis, hepatitis such as chronic, acute hepatitis, and infectious mononucleosis, alcohol liver disease etc. these diseases raises the limit than normal limit nearby more eight times. Again these diseases are non-specific and they may occur in the form of any one or more diseases of then liver. The highest increases occur in diseases associated hepatic cell injury extensively. The hepatic injuries occurs due to viruses, exposure of hepatotoxins such as carbon tetrachloride and paracetamol
etc. ALT is somewhat higher than AST. The increases the aminotransferases can be diminished under certain circumstances. The drugs like increased aminotransferases values if older tests are used for colorimetric estimation. Low concentration of aspartate amino transferase may present in uremia. These values increase after dialysis.

1.2.4: DRUG-INDUCED HEPATOTOXICITY:

The main function performed by liver is metabolism of drugs and compounds which are given orally. The compounds which are metabolized by the liver are mostly soluble in fats and lipids and again they passes through intestinal cell membrane and after passing through the cell membrane and they go through the process of excretion by converting into water-soluble compounds and excreted through the urine.

All the metabolic processes of drug or compounds occurred in the liver by the system of microsomal fraction. Smooth endoplasmic reticulum is the microsomal fraction of the liver. The metabolism of drugs or compounds in liver is by the enzymes like cytochrome P_{450} enzymes & cytochrome C reductase enzyme and they are catalysed in the presence of a co-factor such as Reduced NADPH which increases the activity of these enzymes. The drug polarity is increased by hydroxylation (in which addition of \(-\)OH group) or oxidation (addition of O2). And this polarity is further raised by conjugation or combination of several substances such as glucuronic acid, sulphuric acid etc.

By the administration of drugs into the liver, there is enlargement of liver is occurred which relates to enzyme induction. Para-amino salicylic acid is the inhibitor of the enzyme system. Several active drugs which may be compete for the binding site of enzyme which takes the more time or slow process for catalyzing the reactions or the drugs which take more time to metabolise.[85].
### INORGANIC AGENTS

Metals and metalloids: zinc, thallium, bismuth, antimony, chromium, phosphorous, arsenic, beryllium, boron, cadmium, selenium, cobalt, copper, iron, lead, mercury, gold, tellurium, manganese, selenium, , hydrazine derivative iodides.

### ORGANIC AGENTS

**Natural:** Plant toxins
cycasin, nutmeg, tannic acid, Albitocin, icterogenin, pyrrolidizines, saferole, indospicine.

Mycotoxins:
Aflatoxins, cyclochlorotine, ethanol, luteoskyrin, griseofulvin, sporidesmin, tetracycline and other antibiotics.

Bacterial toxins:
Exotoxins (endotoxins, C.diphtheria, ethionine, Clostridium botulinus.)

**Synthetic:** Non-medicinal
Chloroaromatic compounds Haloalkanes and haloolephins, Azo compounds, Nitroaromatic compound, Nitroalkanes, Phenol and derivatives, organic amines,. Various other organic compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INORGANIC AGENTS</strong></td>
<td>Metals and metalloids: zinc, thallium, bismuth, antimony, chromium, phosphorous, arsenic, beryllium, boron, cadmium, selenium, cobalt, copper, iron, lead, mercury, gold, tellurium, manganese, selenium, , hydrazine derivative iodides.</td>
</tr>
<tr>
<td><strong>ORGANIC AGENTS</strong></td>
<td></td>
</tr>
<tr>
<td>Natural:</td>
<td>Plant toxins</td>
</tr>
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</tr>
<tr>
<td>Synthetic:</td>
<td>Non-medicinal</td>
</tr>
</tbody>
</table>

**Table No. 1.2: Types of Hepatotoxic Agents**

**Mechanism of drug hepatotoxicity:**

The Drugs which are shown damage of liver by a directly react on the liver cell. The drugs are having two other mechanisms are usually involved. The first one is combined with the
proteins cells and which are indicated related to the metabolite substances and second mechanism is that which is modified the immunological reaction are increases to the drug concentration of hepatocytes. Sometimes the liver damage or the injury of liver shown when the hepatotoxic drugs are taken such as paracetamol. The immunological reactions are sometimes predominant when the drugs like anaesthetics such as for halothane.

**Direct action (metabolite – related):**

The hepatic enzymes that takes important part in the metabolism of compounds or drugs and these enzymes make the drug chemically active and stable to the drugs and convert them into more potent agents like acylating agents, arylating agents, alkylating agents etc. For the life of liver cells and liver necrosis are essential to the combination of covalently to the liver of the macromolecules. The liver generally included the substances, like glutathione, which has a capability of more preferentially together with a toxic metabolite. If storage of these components can be removed the necrosis of the liver.

**Immunological reaction for the drug:**

Generally small or some part of liver is affected i.e about nearby 1%. and those drugs causes liver injury. Skin rashes, fever are the symptoms of immunological reactions. But generally children are not affected for that.

The diagnosis may be only being confirmed with the help of drug challenges. The diagnosis is ethically or un-justified when the the original reaction has been a serious one.

**Toxicity of Individual Drugs:**

**Carbon tetrachloride**

It may be administered into the body accidentally or it may be taken for suicidal condition. It is very toxic to the body. During filling of fire extinguisher or for dry cleaning when it is used or when it mixed into the drinks it may be inhaled into the body. Because of the toxic metabolism of microsomal which combined with the covalently with the proteins cell are induced the liver injury and liver necrosis. The after reduction of protein which are decreases the drug metabolism of enzymes and induced effect are enhanced by alcohol, barbiturates etc. These types of effect which are shown under-developed countries and the hepatotoxic effects due to the carbon tetrachloride.

**Tetracyclines:**
Chlortetracycline and oxytetracycline interfere with protein synthesis. In 1963, to cure the pyelonephritis the larger dose of tetracycline is given i.e 3-6 g per day intravenously to the six women during pregnancy in the third trimester. When failure of hepato-renal system, death occurs. Tetracycline may be inhibitory to protein synthesis particularly affecting the transport lipoproteins which remove triglyceride from the liver. Hepatotoxicity occurs when large doses of tetracyclins are intravenously administered during pregnancy.

**Paracetamol:**

For hepatic necrosis paracetamol is a best agent i.e the dose near about 10-12 g is necessary. But the administration of paracetamol is very difficult because it gives immediate unreliable histories and vomiting. Most preferred drug for paracetamol is Glutathione is always combine with the electrophonic metabolite of paracetamol. Glutathione when causes hepatic necrosis the metabolites of paracetamol arylates essential nucleophilic macromolecules.

**Methotrexate:**

It is first metabolized to 6-mercaptopurine. The Hepato-toxicity includes hepatic fibrosis and hepatic cirrhosis occurs when a toxic metabolite of microsomal origin affects on the liver.

**Isoniazid**

The hydrazine is a inhibitor associated with hepatotoxicity which is a weak amino-oxidase inhibitor. This amino oxidase inhibitor used individually in asymptomatic person which has a tuberculin skin tests. During the starting six months of starting isoniazid, in Washington there are a lot of government employees which suffered from liver diseases like fibrosis, cirrhosis. All of that total thirteen persons were suffered from jaundice and two were died. Hydrazine forms when acylation of isoniazid occurs. This formed hydrazine is converted to acylating agent which is more potent by drug metabolizing enzymes, which gives necrosis.

Isoniazid → Acetyl isoniazid → Acetyl hydrazine → acylating agent
Liver cell necrosis

**Halothane**

Convincing evidence has come from challenge experiments such as that in an anaesthetist who developed clinical, biochemical and hepatic histological deterioration when rechallenged with halothane. Two controlled prospective clinical trials have been reported. The first, from Oxford, reported liver function tests in women receiving multiple anaesthetics for the treatment of cancer of the uterine cervix. The second, from Southampton in addition studied men receiving
multiple anaesthetics during the treatment of cancer of the bladder. In both there was an increased incidence raised transaminases values in those receiving halothane.

When metabolism of Halothane is occurs then there is a formation of halogens like chloride, bromide and also trifluoroacetic acid. The formation of the first product of reductive metabolism is unstable and toxic and again they covalently bound to the metallic enzymes of liver which gives the injury to the liver directly.

**1.2.5: LIVER FUNCTION TESTS:** [89,37].

**Test for formation and excretion of Bile**

**1. Bilirubin:**

Bilirubin pigments can be detected in serum, faeces and urine. Serum bilirubin estimation is based on van den Bergh diazo reaction by spectrophotometric method. Diazo reagents consist of diazotized sulphamic acid. Water soluble conjugated bilirubin is determined by van berg reaction with diazo reagent, whereas alcohol soluble unconjugated bilirubin by indirect van den Bergh reaction. In faeces, excretion of bilirubin is assessed by inspection of stools, clay-coloured stools due to absence of faecal excretion of the pigments indicates obstructive jaundice. In urine, conjugated bilirubin can be detected by commercially available dipsticks, Fouchet’s test, Foam test or Ictotest tablet method.

**Urobilinogen:**

It is normally excreted in the urine. Its semiquantitative estimation in the urine can be done by preparing dilutions with Ehrlich’s aldehyde reagent or by dipsticks method. An increase in urobilinogen in the urine is found in hepatocellular dysfunctions such as in alcoholic liver disease, cirrhosis and malignancy of the liver.

**Bile Acids (Bile salts):**

In the hepatic cells or liver cells, the cholesterol synthesizes two acids namely cholic acid and cheno-deoxy cholic acid which are termed as the primary bile acids. The secondary bile acids synthesized when these bile acids like cholic and deoxy-cholic acids on secretion into the gut come in contact with colonic bacteria and undergo deconjugation. When there is an increase in concentration or level of bile acids of the serum, the hepatobiliary diseases are associated with cholestasis and these diseases produces itching. In the hepatobiliary diseases like cholestasis, the excretion of bile acids are through the the urine by the mechanism of passive
diffusion and active transport and these bile acids are confirmed by simple methods as hay’s test and dipsticks.

1. **Serum Enzyme Assays:**

   The estimation of several serum enzymes are most commonly used in various types of liver injuries, whether hepatocellular or cholestatic, as well as in quantifying liver damage. A combination of serum transaminases and alkaline phosphatase estimation adequate to diagnose liver injury.

2. **Alkaline phosphatase:**

   The enzyme like Serum alkaline phosphatase is synthesized from number of organs and tissues like intestine, liver, specially from bone, and placenta. Elevation of enzyme activity may be indicated in various diseases of liver, bone, and also associated in pregnancy.

3. **Glutamyl transpeptidase:**

   The primary source of the enzyme, in serum is the liver. Its serum level parallels serum alkaline phosphatase and these enzyme detect that there is an increased level of an enzyme serum alkaline phosphatase is from the hepatobiliary origin. If the increased level of Glutamyl transpeptidase in hepatobiliary diseases like cholestasis and hepatocellular disease, it is present in high concentration in those patients which are with alcohol abuse but there is no liver damage.

4. **Transaminase (Aminotransaminase):**

   The Serum Aminotransaminase which are plays important role for estimation of the hepatocellular injury and it is most commonly used in detection of the acute hepatocellular disease like as a hepatitis. These enzymes catalyzes the transfere of the keto group of the ketoglutaric acid and amino group of the amino acid which forms respectively the oxaloacetate and pyruvate from by SGPT and SGOT.

**1.2.6: THE ROLE OF CYTOCHROME P<sub>450</sub> IN DRUG-INDUCED LIVER DISORDERS:** [27,86].

The chemical-induced liver which are generally not shown the result from the effects of the original compounds. The conversion of the original compounds to toxic metabolites within the liver. This statement applies not just to drugs, but also to environmental chemicals such as
aflatoxins, bromobenzene and carbon tetrachloride. The major families of liver enzyme which are produced they are responsible for the generating potentially toxic metabolites which are from the cytochromes P₄₅₀.

**The role of cytochrome P₄₅₀s:**

The most of drugs are capable of producing liver toxicity appears through the generation of toxic metabolites which are present in the liver. The important role of metabolites in chemical-induced injury probably reflects the fact that in order to be absorbed into the body and reached the liver, chemicals must generally be lipophilic and metabolically stable. Metabolism of drugs which are generally produces metabolites that are safely removed from body. However, under the certain circumstances P₄₅₀s can be generated most potential and reactive toxic metabolites. The major enzymes responsible for the production of hepatotoxic metabolites are the P450s. Species differences in P₄₅₀ catalytic activity and regulation probably contributes to the imperfect animal studies to identify hepatotoxins.

For Example-Acetoaminophen is the compound which believed to cause the toxicity in the liver occurs the production of the substance like n-acetyl benzoquinone amine metabolite (NAPQI). The Studies include with recombinant enzymes of human liver suggested that this reactive and potential metabolite could be produced by several cytochrome.

**Carbon tetrachloride (CCl₄) induced hepatotoxicity** [82,90,83].

The Carbon tetrachloride (CCl₄) is considered as a very toxic and powerful agent to the liver and this is is used for the induced or damaged the liver which is useful for the observation of liver necrosis as well as liver steatosis in the rat. These free radicals are produced cytochromes P-450 mediated which are reductive process. During the process the carbon tetrachloride is converted into (CCl₃) and (CCl₃O₂). These radicals which are reactive and their time of action is very short carbon tetrachloride created necrosis is most severe in centrilobular hepatocytes during this process the concentration is high of cytochrome P-450. As per the above observation the free radicals which are important for the produce lipid peroxidation of biological membranes, protein binding, covalently to lipids and nucleic acids. Others are considered that the trichloromethyl radical which are important for the lipid peroxidation and covalent bonds.
Fig. 1.5: Sequence of hepatotoxic effects following lipid peroxidation induced by free radicals formed in carbon tetrachloride metabolism
Toxic injury of liver induced by carbon tetrachloride (CCl₄) is a model system of toxic injury. Toxic injury of liver by CCl₄ is the result of their metabolic conversion by a complex of enzymes bound to membranes of the smooth-surfaced endoplasmic reticulum. Action of these enzymes is a major mechanism by which toxic compounds are converted to less toxic ones. In some instance non-toxic substances are metabolized to toxic ones such as in case of CCl₄.

Carbon tetrachloride is converted by hemolytic cleavage to a highly reactive haloalkane free radical and a chlorine free radical in the following reaction:

\[ \text{CCl}_4 \rightarrow \text{CCl}_3 + \text{Cl}. \]

These in turn react with a variety of intracellular molecules, notably the unsaturated fatty acids.

Polyenolic fatty acids for example are converted to organic free radicals which in turn react with molecular oxygen to form organic peroxides. These compounds are highly unstable and decompose spontaneously to form aldehydes, ketones and other products.

CCl₄ reacts with sulfahydryl groups which mediate the function of the many cell proteins including a number of important enzymes and this reaction leads to their alkylation and subsequent loss of function. The free radicals formed react rapidly with other molecules to form additional free radicals, such reactions are autocatalytic and tend to spread from a small focus to involve large areas of cytoplasm. The earliest change that has been detected in rat liver cells is a functional one that occurs after the 30 min of the administration in to the git. in a dose of a 0.25 ml(single dose) of CCl₄. It consists of rapid decrease in synthesis of the export protein albumin as well as the cytochromic. The diminution of protein synthesis after intoxication appears to be linked to disaggregation of the polysomes and probably represents a physical disruption of their association with messenger RNA.

Significantly in this early phase of carbon tetrachloride induced injury, mitochondria appear morphologically intact and are capable of normal oxidative phosphorylation and fatty acid oxidation among their many functions.

Within few hours after administration of CCl₄ neutral lipids (triglycerides) begin to accumulate in the cytoplasm making their first appearance as osmiophilic droplets ultimately fill the entire cytoplasm. Approximately 10 to 12 hours after CCl₄ administration the liver is grossly
enlarged and pale because of accumulated fat. Lipid can also accumulate in the liver by mechanisms such as by increased mobilization of free fatty acids from depot fat.

Shortly after the ingestion of as little as 5 ml to as much as 100 ml of carbon tetrachloride, swelling and hydropic degeneration of the centrilobular hepatic cells develop. These changes progress to a diffuse fatty degeneration and necrosis in the centrilobular parenchyma with collapse of the reticulum network, followed shortly by haemorrhage and leukocytic infiltration.

Autoradiographic studies have shown a rapid uptake of carbontetrachloride by the cytoplasm and nuclei of the cells of centrilobular areas. Autoradiographic avoidance shows that radioactive 14C and carbon tetrachloride remain in the centrilobular areas as long as 2 days after ingestion.

Endoplasmic reticulum is damaged 30 minutes of the administration of carbon tetrachloride, whereas the mitochondria survive unaltered for several hours. Protein synthesis is reduced within 2 hours of poisoning. Fatty acids are mobilized from peripheral fat depots to the liver. In the liver cell they are oxidized to triglycerides.

Experimentally, CCl₄ has been widely used to study toxic hepatic necrosis and it is still a favoured model of cirrhosis which regularly develops after repeated injection of CCl₄ into rats.

1.3: STUDY OF HERBAL EXTRACTS:

1.3.1: INTRODUCTION TO HERBAL EXTRACT:
- Extract are concentrated preparations of solid, liquid or intermediate consistency usually obtained from plant or animal matter.
The extraction of drug was prepared by extracting herbal drug of suitable particle size with suitable extraction medium (menstrum).

There are various methods which are used to complete extraction process. The liquid getting after extraction of drug and the drug residue is known as micelle.

For the preparation of medicinal preparation can be micelle. Example- fluid extracts and tinctures.

Some terms used in extraction:

- **Mestrum**: The solvent or solvent mixture used for extraction.
- **Micelle**: The micelle means solution containing extracted substances.
- **Rinsing**: The dissolution of extractive substances out of disintegrated cell.
- **Lixivation**: The extraction with water as solvent or linching.

1.3.2: CLASSIFICATION OF EXTRACTS:

1. Aqueous drug extracts: a) decoction b) infusion c) maceration
2. Tinctures
3. Fluid extracts
4. Thin extract
5. Thick or viscous extract
6. Dry extract
7. Oily drug extract
8. Oleo resins extract

1. Aqueous drug extracts:

These are medicinal preparations as dispensing medicine intended to be used immediately after preparation of medicine and also important to be preserved for specified period. There are different methods are generally used for the preparation of extract.

- The crude drug and menstrum are transferred in closed container and shaken occasionally for seven days.
- Stain press the murc, clarify by filtration
- Filtrate is not adjusted volume (organized drug).
For unorganized drug, the drugs shaken occasionally with 80% of menstrum for 2-7 days in closed vessels. Supenentant liquid is decanted and merc is not pressed rest of the menstrum is passed through the filtred.

2. Tincture:

The alcoholic preparation called as tincture. These are the extracts obtained from plant or animal drugs by using varying solvent such as ethanol with certain expipient in such a way that one part of drug is extracted with two to ten parts of menstrum. The solution of dry extracts in ethanol of suitable concentration are also regarded as tinctures.

3. Fluid Extracts:

These are tincture like liquid preparation. These are more concentration and as per recommendation of standdared text, two parts of liquid extracts is made from one part of drug.

4. Thin Extracts:

These are also known as extracts tennua. These are prepared as liquid extracts but concentrated to honey like consistency.

5. Thick extract or viscose Extracts:

These extracts are thickly liquid or viscous when warm. During the warm of extracts kept at room temperature and obtained from micelle by extensive but take a care about concentration. They are plastic masses containing varying quantities of recedual moitures and are susceptible to microbial growth, hence replaced by dry extracts.

6. Dry extracts:

These are plant preparation extracts are obtained by drying of liquid extracts.

7. Oily Drug Extracts:

These preparations are prepared by drug material in to oil adapting maceration process. For the drying of oil drug extracts lightly heat also be used for removale moisture which are presence in the drug extraction in short duration. The drugs are considered for oily preparation are henbane, aconite, marriegold, chamomile flower, rose flower.

8. Oleo Resins:

These are preparations made by extracting oleo resins material like plant gum and resins from species with suitable solvent like ethanol, ethy acetate.

PROCESSING:

During the processing of extraction the following important steps are used-
a) Pulverization.
b) Extraction.
c) Purification of micelle.
d) Concentration of micelle.
e) Drying of extracts.
f) Pulverization of dry extracts.

Most of the crude drugs are usually put in quarantine store and the remaining drugs store in QA. During storage proper ventilation, humidity controls, suitable temperature and light condition should be ensured to maintain their pharmacological action intact. The crude plant material being taken for processing, should be analysed for following tests as per USP.

a) Macroscopic and Microscopic examination for identification.
b) Examination of foreign organic and inorganic impurities.
c) Determination of LOD, moisture contents, ash value, extractive value.
d) Determination of microbial contamination and absence pathogenic bacteria.
e) Examination of pesticide residue.
f) TLC examination.

**EXTRACTION:**

The extraction of drugs is represents separation of solid from solid. The solid substances should be extracted from solid components. The solid-liquid extraction are generally known solid-liquid and the solid crude drug is extracted with a liquid. For the extraction of plant materials having two processes these are following.

1. The rising of extractive substances out of disintegrated plant cell
2. The dissolution of extractive substances out of intact plant cell by diffusion.

This process requires swelling and steeping of drug plant materials in order to increase the permeability of cell wall.

Various extraction processes commonly used by different manufacturers are classified as-

i. Maceration
ii. Hot continuous extraction
iii. Percolation
iv. Supercritical fluid extraction
v. Counter current extraction
vi. Seam distillation
vii. Vertical extraction
viii. Column extraction

1. **Hot continuous extraction:**

The use of Soxhlet Extracter is a convenient way to prepare crude plant extract. This procedure is used mainly with pure solvents. The main advantage of Soxhlet during the extraction is automatic and continuous method that does not require any manually arrangement apart from concentration of the extracts and prevent the loss of solvent by recycling it over the sample.

Dis-advantage is that the extractives are heated during the time of extraction the boiling point of the solvent of the loss and thermolabile compounds like cariotionides may hydrolised or decompose.

In this method the air dried powder material to be loaded in a tube of soxhlet body tube also called as thimble tube which is prepared from cellulose central compartment which are connected to siphoning tube and side arm tube connected to below neck of Soxhlet apparatus and reflux condenser is connected to the Soxhlet apparatus.

The solvent in round bottom flask is heated to boiling and vapoure are passesed into the condenser by the the side arm of the soxhlet apparatus. With the help of condenser the vapour are condensed to form liquid and dripes in to the thimble containing the material which are going to extracted. By the use of a material the warm solvent strained and the extract gradually collectes in the central compartment. Ones the height of the extracts reached at the upper part of the siphon tube i.e the top side, then the liquid present in the middle compartment of the siphon tube is flow throughout the siphon tube and again comes down to the solvent which is present at the lower side. This process continous and repeated until the extract collects in the lower vessels, gradually becoming more and more concentrated.

The extraction method with the help of Soxhlet apparatus is most usefull in the plant material extracts by using the var for the extraction of plant material with specifious extraction solvents like chloroform, petether, ethanol, methanol, etc.
If constituents are nearly soluble in menstrum or penetration of cellular tissue by menstrum is very slow drugs are extracted by continuous heat extraction processes example cantharidine from Cantharides, alkaloid from seeds and extract of fixed oil.

2. Stem distillation:

It is a popular method for the distillation of volatile oils for its purification without any decomposition of its. In this the volatile oils are extracted from the plant material. In this two immiscible liquids are heated together up to boiling. When there is vapor pressure is equal to the atmospheric pressure then the liquid starts to boiling. In this the mixture boiling point is less than that of the liquid with less boiling point. The plant material which are used for extraction are taken with water and heated up to boiling. During the boiling of the liquid when steam coming from the safety tube and these vapours get collected and condense it. again the oil is get separated from the water. The steam distillation unit consists of a steam can which is fitted with a cork which have two holes. One hole is connected to a flask containing non aqueous liquid and another hole is for safety tube. It also consists of condenser, adaptor, bent tube etc. Steam distillation method is useful for the preparation of volatile oils, to determination the percentage of volatile oil in the drug, for purification of volatile oils.

3. Supercritical fluid extraction:

The critical point of means the pure substance is known as the highest temperature and pressure which are presence in substance in vapour liquid equilibrium. The supercritical is that, the pressure and temperature above the critical point a single homogeneous fluid is formed.

Supercritical fluid can dissolve a wide variety of organic compounds there solvent power can be different about there critical points when the low pressure and temperature changes.

Supercritical fluid extract has many advantages which are as follows-

- It uses small amount of solvent.
- Its selectivity in control

It needs less degradation and clean extracts in compare to steam distillation and conventional solvent extraction.

Supercritical fluid posses’ superior mass transfer properties by virtue of there low viscosity and high solute diffusivity along with the ability to penetrate microporous materials. Supercritical CO₂ with its particular analytically attractive properties such as non toxicity, non inflammability, non
corrosive ness, chemical inertness, low critical temperature (30 °C), moderately low critical pressure (73 atm.), easy availability, cost effectiveness and environmental exceptibility is the preferred solvent for many supercritical extraction.

4. **Percolation**:

This is official method of extracting drug used in preparation of tincture or extracts the process has three stages

i. imbibation

ii. maceration

iii. percolation

Process of imbibation is nothing but moistening drug under percolation with menstrum by aloning drug to stand for 1-4 hrs. constituent during this drugs swells and menstrum penetrats the cell wall to dissolved chemical constituents more readily. During maceration drug is kept in contact with menstrum for 24 hrs. witle conh occasionally shaking where in menstrum gets saturated with solublconstituents of drugs. Thus percolation is process where maceration is followed by downward displacement of sat rated menstum, and drug is existed by slow passage of menstrum through the collom of drug. For this perposs imbibed material is packed uniformaly and heatally. Uniform packing insures uniform run of menstrums and efficints extraction so, drug is placed layer by layer which will not disturbed even suficint menstrum is added to percolates. Material of percolator should be in constant contact with sufficint quantity of menstrum to avoid craking of bead.

Percolator made of glass and steel may be used if pecolation is to be carried at elevated temperature it should be provide by steam jacket.

Open type percolaters can be used if water is menstrum and closed type for alcohol, ether etc.

5. **Vertical/ turbo Extraction**:

In this process plant material to be extracted is stirred in menstrual with a high speed mixture or homoginiser. The shearing process breakdown the drug material to a partical size less than that of material. The diffusion of extractive substances through the cell membranes is largally replaced by washing out from the destroyed cellular tissue and this result in substantially faster establishment of the maceration equalibriun in considerably shorter time. The energy supplied for high speed sturring and communication may some time raise the temperature.
During extraction which may be desirable because of the risk of decomposition of the volatile constituents this can be controlled and process can be carried out at any desirable temperature by direct cooling or by stopping the process from time to time. Example higher recovery of active constituent of belladonna, hyocyamus, cinchona bark.

6. **Counter current Extraction**:

   In continuous counter current extraction, a moving material solution, emulsion or solid mass is extracted by liquid phase following against it. For extraction of herbs, the material put in the extraction plants where it advances on moving bead and comes in contact with extrether baction solvents. Further the material is moved in extraction plat, which is coming in contact with the fresh solvent that is most concentrated solvent comes in contact with fresh hearbs and fresh solvent comes in contact with exohsted hearbs. The most logical extention of counter current fasion is continous in which the herbs are charged at feed end of continous jacketed extracter and are conved further by a slow moving screw till it charges to the exhaust end. The exhausted hearbs from continous extraction are squeezing in reducing pitch screw and finally dumped in solvent recovery vessels where it is subjected to vaccum to recover the solvents. Examples - Helical counter current extracter.

7. **Column Extracter**: In which the hearb material is extracted with and excesses of solvent in a counter flow apparatus with built in columns.

8. **Ultra Sound Extraction**: The ultra sound frequency of 20-2000 kHz. has been used in extraction. The principle effects of this type of extraction are to increase the permeability of the cellwalls to produced cavitations. Altrsound extraction has so far not widally used on a large scale because of high energy cost. Example- belladonna hearb, Rauwolfia root, ipecacunha, datura etc.

**PRE EXTRACTION PROCETURE:**

- Before the herbs are taken for processing, we have to be sure that it is correct drug. This should be down with quality controle release slip attached to bag by quality control.
- Reducing the partical size of herbe which are perfect for the extraction. If the particale size of drug are more than required for the extraction, the extraction will not complete properly. If size of course powder is less than required size of particals, there will be chances loss of solvent. so, and extraction agained will not be complete. Generally three sizes are required for extraction course powder, medretally course powder and fine powder.
The herb should soaked with the solvent to be used, prior to extraction for a period of 8-48 hrs. as may differ from herb to herb. If the herb having hard fiber it takes the more time for preparation of soften.

**CHOICE OF SOLVENTS:**
Selection of solvent is made on the basis of –
- Pharmacopial guidance, if the herbal extraction is given in prescribed pharmacopeias then we can strate away use the same solvents in our work.
- If pharmacopial guidance is not available, then, go through the review of literature of active principle which are present in the plant.
- In some cases used particular solvent for the isolation of active ingredient from a herb as compaire to all active ingredients in the total extracts.

**TEMPARATURE:**
During the extraction obtained from the plant sources the temprature should maintained about 80 ºC in any case but it should preferabally near about 60-70 ºC occasionally, lower temperature upto 50 ºC may be also required depending upon the herb and purpose of extraction.

**FILTRATION:**
Filtration may be defined as the the process which are separated solid materials from liquid. There are various filtration are available. The filtration equipments can be selected from the following option-

a) Nutch Filter: These types of filtration is the simplest and most in expencive type of filter which uses the driving force created by full vacuum and also provides a storage volume for filter liquid. However, such filters are used for small scale application only.

b) Enclosed filter press: This filter is continous and capacity is no limitations. Thesolid wast separated and stored in between two plates or the filter press. The filtrate is lead through the piping to the storage tank.

**EVAPORATION / DISTILLATION:**
The Evaporation means as the removal of liquid from a solution by bolling the solution in a suitaible vessel from where the vapours are withdrawn and a concentrated semi-solid recudue are left. Evaporation is maimum at the bolling point of the substances.
Distillation is process of the separating the constituents of a liquid by vaporinsing the liquid and passing to a liquid. Therefore, this methods plays important role to change of state from a liquid to vapour and again converted in to liquid.

The filtered liquid has only 2-5 % dissolved solid which are extractrd from hebs. The concentration has to be increased by evaporating and distilling the solvent from the filtrate.

- When the solvent is water, the evaporation can be down in jacketed pan and no solvent recovery is required.
- For solvents which are inflammable and expensive the jacketed vessel has to be enclosed and solvents are lead to a water cooled condenser and the condeset is stoered in enclosed reciver.
- Vaccumme is applied for enhancement of rate of evapoaration rate without causing detoriation of heat sensitive materials.
- The capacity of equipment can be further enhanced by providing a well digned agitater which not only create turbulence in the mass but also reviews the thick films of the materials adheriing to the sides of vessels.
- The recidence time of the materials in the vessels is from 8-24 hrs., causing slight detoriations in quality of heat sensitive material so, care should be taken.
- A better system which gives equalent capacity of evaporation with minimum residence time of the materials such facility are offered by continous double stage evaporates working on the principle of mechanically agitate evaporaters, thermoshiphon evaporaters, falling film evaporaters etc.
- The final selection of a evaporaters depends on the capacity, properties of feed products like frothing, sticking tendancies, viscosity, heat sensitive charactrecstic etc.
- Froth breakers and anti foaming agents are also added to create the desire effect.
- The extracts are concentrated in evaporation equipments to 30-35 % solid contains

**SOLVENT RECOVERY:**

This is done by providing solvent recovery system bychilling the vent gases from the system to 5-10 C by passing them over refrigerater vent condenser. Due to reduction saturated vapoure pressure at such low temperature, the solvent condences out in the vent condenser. The recovery solvent is the net advantages in the economics of the herbs processing.

**DRYING OF EXTRACTS:**
The extracts from evaporation / distillation system are fed to suitable drying equipments. There are a number of options available to select from depending on the capacity, heat sensitive properties etc.

Drying is defined as a process in which the liquid is removed from a solid compound by vaporization with the help of heat. The equipment which is used for drying purpose is known as dryer.

In pharmaceutical industry as well as in laboratory, there are various non-thermal methods and equipments are available for the purpose of drying, the choice of which depends on the following factors:

- Whether the product is sensitive to heat or not.
- Physical characteristics the product before drying.
- The nature of solvents to be removed
- Whether the process to be carried out under aseptic conditions or not.
- Quality of the product to be dried.
- Available source and heat.
- Cost involved.

**Types of dryers:**

1. Dryers for dilute solution, suspension & slurries
   - i. Drum spray dryer.
2. Dryers for damp solid materials include:
   - i. Tray dryer
   - ii. Tunnel dryer
   - iii. Rotary dryer
   - iv. F.B.D (fluidized)
   - v. Vacuum dryer
   - vi. Freeze dryer.
FACTORS AFFECTING HERB QUALITY:

Due to the increasing demand for herbal medicine in Global drug market, and in the developing as well as developed countries which are increasingly maintained the material quality as well as material purity and finished compound or products. Even today we are facing the standardization problem which are relating to herbal drugs arises from the mixture of drugs which are obtained from parts of plants as well as whole plants or formulation.

To check the quality of herbal remedy and specific type of control of raw materials which are using is very important. For controlling the quality of the raw material, the following aspects are need to be considered.

1. Authentication and reproducibility of Herbal ingredients:
Herbal ingredients must be accurately identified by macroscopical and microscopical characteristic in comparison with authentic materials or accurate description of authentic herbs. It is essential that herbal ingredients correctly authenticated, it is important to realized that different batches of the same herbal ingredients may differ in quality due to a number of factors.

2. Inter and intra species variation in plants:
The primary and secondary metabolites varies considerably if there is a inter and intra species variation in different plants. This results in variation in the individual constituents and thereby causes difficulties in standardization. These all variations are genetically controlled which is related to the country of origin for that particular species.

3. Environmental factors:
Environmental factors like climate, altitude, rainfall, and other conditions are responsible for the growth the plants, which in turn affected the quality of herbal ingredients, present in a particular species, even if it is in the same country. This results in major variation in the herbal ingredients present in some specific species of plants.

For Example:
The formulation of volatile oil appears to be high at higher temperature, even through high temperature lead to an excess ona is cultivated in lower altitude less amount of alkaloids are
present. Excessive rainfall also leads leaching of volatile oils, alkaloide or glycoside from the plants.

4. **Plants part used:**

   Usually the active constituents vary between different parts of a plants. The parts of the plants, which is not normally used, are adulterated with the herbal ingredients. Sometime the exhausted plants parts of the same physical appearances are mixed to increases the weight of herbal ingredients.

5. **Time of harvesting:**

   Collection of a particular herbal ingredients is done only after the optimum time of harvesting which is specified for that drug. This is because these constituents obtained from secondary metabolites, vary considerably during growth cycle. Hence the quality of the drug and concentration of the constituents depends on the proper time of harvesting.

6. **Post harvesting factors:**

   After harvesting herbal raw material are undergoes drying, storage, transport etc. which also can greatly affects the quality of the herbal ingredients. Improper storage will leads to the microbial contamination. Similarly the processes like drying may results in a loss of thermolabile activity constituents. Example, the excessive moisture facilities enzymatic reaction resulting in decomposition of active constituents of Digitalis leaves and wild cherry bark.

7. **Contamination of Herbal ingredients:**

   The presence of contamination like soil, insects, other animal matter and excrete will also affected the quality of the drug. Some pathogenic organisms like Enterobacter, Entrococus Clostridium and Pseudomonas etc. have been proven to contaminate different herbal ingredients.

8. **Pesticide, Fumigates and other toxic material:**

   These may affect quality of the drug which are applied during cultivation, storage etc. which included DDT, Organophosphate, Ethylene dioxide, lead, cadmium etc. limit tests for all these pesticide, fumigates and other toxic materials is necessary to controle the quality of the plants materials.

**QUALITY CONTROLL & STANDARDIZATION OF HERBAL DRUGS:**

The **QUALITY CONTROLL & STANDARDIZATION** is very important thing for herbal drugs. Nowdays World Health Organisation recommending, promoting, encourage treatments by using
herbal drugs or plants in natural health care programme, because these drugs are easily available at low cost, very safe and more over people have faith in these remedies. In global market the drugs which are derived from plants materials and herbal preparation have a substantial proportion. Therefore for the standardization and assessment of quality, the guidelines are followed which are recognized by international level.

Therefore the WHO recommended that use the various modern techniques and standards which are suitable to assess the quality of drugs derived from medicinal plants. To the pharmaceutical purposes the quality of medicinal plant materials should be as high as compared to the medicinal preparation.

If the raw material used and stage-by-stage process of manufacture are standardized then the final product is expected to be confirm to uniform standards.

A. Major difficulties in Standardization of Herbals:

1. Uncertainty about activity constituents:

Herbal materials are chemically complex in nature as it consists of several constituents. Standardization of these active constituents of the drugs does not reflects reality, because the biological activity is not exclusively depends on one active constituent but is due to the overall chemical constitution of the plants.
Though the other inert components do not directly affect the pathological mechanism, but their presence may influence the bioavailability & excretion of the active constituents. Sometimes the stability of the active components may be increased & the rate of side effects may be reduced by these inert constituents.

If different active components are present in plant they may have additive or synergistic effect since the biological activity depends on several components, it is not possible not optimize all of them during quality control.

2. Inconsistency of Chemical Composition:

The chemical composition of the medicinal is inconsistent because following factors may alter the chemical composition of the raw materials.

- Agro climatic condition.
- Geographical variation.
- Natural association with other plants.
- Harvesting time.
- Post harvesting handling
- Storage of raw materials
- Size reduction, drying & extraction operation.
- Manufacturing processes
- Equipments used
- Storage of finished products.

So eliminate some causes of inconsistency one should use cultivated rather than wild plants.

Other difficulties include the presence of substituent’s & adulterants, toxic plant constituents, contamination due to pesticides, heavy metals, microorganism, and fumigation agents.

Hence, during quality control of medicinal plants, complex nature & inconsistent composition are also considered. Moreover we should realize that herbal medicines are not like synthetic medicines & they can’t be controlled like synthetic medicines. So quality control of herbal medicines needs different approach than that of synthetic medicines.

B. Current approach in standardization of herbal medicines:
Presently modern analytical methods like chromatographic techniques like TLC, HPTLC, and HPLC & GC etc are used for quality control of herbal materials & medicines.

Herbal materials are evaluated by using chemo profiling of the plant. Chemo profiling of the plant & its relation with the biological activity established by the following way-

- If the active constituents which is present in the herbs, has the recommended therapeutic activity then they are considered as marker compounds. And used for the standardization of tests herbs. example in Ashwagnadha concentration of active ingredient withholds are use marker for standardization.
- If the active constituents are not known then the compound which is present abundantly are used as marker compounds for standardization. example in Aegle marmelos, aeglin is used as marker, even though aeglin is not linked with the recommended therapeutic activity of Bilva.
- Repetitive TLC for the herbal extract is done, 4-5 compounds which are present predominantly in the TLC, are selected as marker for the standardization. They are isolated, purified & chemically characterized then they are correlated with the biological activity of that herb. In actual standardization, TLC of the standard marker compound is compared with that of TLC of the extract.
- Following all the tests given in the herbal/traditional medicine book.
- Stress on selection of herbs & during manufacture follow validated procedures in the presence of qualified person.
- Use of modern analytical tools for monitoring the process of manufacture,
- Manufacturing should use modern formulating agents & facilities in order to build quality herbal medicines.

In India ICMR – (Indian Council of Medical Research) validated the claims of efficacy of traditional / herbal remedies. RRL regional research laboratory Jammu is the only center established in the country by ICMR for the purpose of formulation, standardization & monitoring quality of such herbal drugs.

C. Development of standardization parameters for assessment of crude drugs according to WHO guidelines:

WHO has given small documents which contains general methods for testing the identity (microscopic, macroscopic, chemical test, TLC finger printing) of the drug. Parameters like
Physical and chemical such as moisture content, ash value, volatile oils determination, extractive value & Thin Layer Chromatography for the purity & quality. Who has given general limit for microbial, pesticidal, heavy metal & radioactive compounds.

1. **Sampling**:

   Before a consignment of a drug can be evaluated, sample must be drawn for analysis. The sample drawn must be the representative of the material which is being undergoing tests. If the sample size is more than 100 kg then around 500gm is taken.

2. **Authentication**:

   The authenticity of a crude drug is established by comparing the plant material collected from the appropriate region of the country, at an appropriate stage of its growth with the description of its given in the pharmacopeia or the other official publications of that country. Example - a number of phyllanthus species like P.amarus, P.madraspatensis, etc. are grown in India in the same agro climatic condition out of which P.amarus has been selected for clinical trials.

3. **Organoleptic Evaluation or Macroscopical Evaluation**:

   Organoleptic evaluation is done by means of organs of sense, which is sufficient to evaluate the drug to be identified. This gives the idea about the quality of drugs. This evaluation includes general appearance, taste and odour of the drugs.

   For Example-
   - In cases of barks, roots, rhizomes, etc shape and size.
   - In cases of leaves types of shape, margin, bars, apex.
   - In cases of flowers like pyrethrum, saffron, types of fluorescence.
   - In case of fruits like cardamom, beal, their size, shape, surface characteristics are the identification features.
   - Taste in case of Phyllanthus amarus, which are bitter in taste whereas P.fraternus is not bitter and P.madrespentensis slight bitter in taste.

4. **Microscopical Evaluation**:

   The Microscopical evaluation of the plant drug is not only helps in the study of the presence of adulterants but also helps in the correct identification of the medicinal plants. For this purpose the drug is soaked in water if it is not fresh then fine transverse section is taken and
stained for the study of the arrangement of the cells important staining liquids used are Phlorogucinol and HCL for lignified tissues, chlor-zinc iodied for cellulose tissues, Ruthenium red for Gums and Mucilage containing cells.

The slide of this test drug are compared with slides of the authentic crude drug description given in the references book. This helps in the study of substances like starch, fixed oil, aleuronic grains, calcium oxalate, mucilage etc. For Example: *P. amaraus* shows wavy walled epidermal parenchyma whereas *P. madraspatensis* shows straight walled epidermis parenchyma.

The study of the surface constants is the important phenomenon of histological evaluation which includes palisade ratio, stomatal index, stomatal number, vein islet number etc. and using camera Lucida. These feature are used for the authentication of leaf drugs and to detect adulterants and also help to differentiate closely related species. For Example- Anisocytic stomata in *P.madrespatensis* whereas both paracytic and anisocytic stomata in *P.amarus*.

Microscopical evaluation is also essential in the powdered drugs. Such diagnostic microscopical feature helps in plants drugs Standardization.

5. **Foreign Matter**:

Other part of the plant or organic matter present in the drug which is not complying with authentic drug may be considered as foreign matter. Other planta may be present due to improper harvesting and garbling. During storage insects, moulds, animal excreta will add to the crude drug. The Medicinal plant material can be entirely free from them, which is difficult. So, Pharmacopoeias provide the limit for the presence of other parts of the plants.

6. **Moisture contents**:

The deterioration of the compound is occurred by moisture content. The moisture content in the compound activate the enzyme and promote the growth of microbes which are responsible for the deterioration. Then these drugs have no economical importance. There are a number of methods which are used to determine the moisture content, these are some physical and chemical methods as follows-

- Spectroscopic method
- Karl Fischer method
- Loss on drying
- Azeotropic distillation method.

- **Loss on drying:**

  The loss on drying of the drug sample is calculated by heating the crude sample in an oven at 105 °C for 30 min. Loss on drying is done for the purpose of removing water and moisture content in the crude drug and to calculate the loss of weight of drug after drying the sample. The weight of the drug sample is increased mainly due to the presence of water and moisture content.

- **Karl-Fischer Method:**

  This is chemical method extensively used for the determination of very small quantity of moisture. For example: the crude drugs like digitalis, Ipecacuanha. The dioxin and aliquot is taken for titration. The Karl-fischer reagent contains solution of Iodine, So2 and Pyridine in dry methanol, Iodine is reduced by So2 in the presence of water causing loss of dark brown color of the reagent.

- **Azeotropic Method:**

  Water forms a heterogeneous azeotrope with the solvents like toluene, benzene and xylene. Such types of binary mixture can be distilled up to the azeotropic composition. Dean stark apparatus is used for the determination of moisture content. Organic solvent forms a azeotropic mixture with water present in the crude drug. When the drug is heated, the organic solvent and water is distilled together which is collected in a graduated tube of the apparatus. Water forms a bottom layer being heavier which can be directly read after complete distillation.

- **Spectroscopic Method:**

  Water will absorb energy at various wavelengths in electromagnetic spectrum this factor is considered as the quantitative determination of moisture, which is done by IR and UV this method ideal for the substances that contains very less quantity of water.

7. **Extractive Values:**

   The biological activity of crude drug is determined by the presence active constituents in that drug. These active constituents are soluble in solvents which are polar as well as semi polar or non polar. If the active constituents are totally soluble in a solvent, this is reffered as
extractive values. Thus to determine extractive values the biological activity of a drug is necessary. According to we can calculate the compound polarity.

The processes like continuous extraction, maceration, pecolation, by using Soxhlet apparatus, are used to determine the extractive values by dry weight or wet weight. For Example: For Ginger, valeriana, Alcohol soluble extractive value For Gentiana liquorice, the water-soluble extractive value. Petroleum ether and ether soluble extractive are determined.

8. Ash value:

After incineration of crude drug, the crude drug removes the inorganic salts which is considered as ash. The determination of ash values indicates the purity and quality of the crude drugs. The ash is present in the compound is in very less quantity. There are various types of Ash value which are determined as-

i) Total Ash:

The total ash in which carbone and organic matter present in the drug. For the preparation of ash form drug is required the temperature 450 °C or above. It mostly contains carbonates, phosphates, silicates and silica. Total ash can be used further to study these are following-

ii) Water-soluble ash:

It is obtained by separating the water-soluble material from total ash, which is then dried to get water-soluble ash. In this method water insoluble material is removed to find the water-soluble ash content.

iii) Acid insoluble ash:

When prepared the total ash it may be treated with dilute Hcl which removes many inorganic salts to yield mainly insoluble ash. The acid insoluble ash which are prepared from Senna, Clove, Liquorice indicates the contamination with earthy materials.

iv) Sulphated ash:

The crude drug is incinerated at a temperature of about 600° C with dil H2SO4 before ignition. This process converts all oxides & carbonates to sulphate salt.

Volatile oil determination

Many Pharmacopoeias recommends minimum standards for the % of volatile oil present in number of drugs. It is determined by steam distillation method by using Clevenger’s apparatus.
Accurately weighed amount of drug is placed in the distillation flask with water or mixture of water & glycerin, volatile oil content present in drug get separated at the boiling temperature of water & gets collected as a layer on the top of water which is then measured in the graduated receiver.

For the volatile oil with relative density more than 1, placing a known volume of xylene in the receiver & reading off the combined oil & xylene makes separation from water.

9. Chromatography:

Pharmacopoeias are increasingly insisting to employ TLC as a mean for evaluating quality & purity. The Rf value obtained by TLC is used as an aid for identity.

\[
\text{Rate of Flow (Rf) = \frac{\text{Distance moved by the solute}}{\text{Distance moved by solvent front}}} \]

The qualitative extracts of crude drug are prepared & are compared with standard or marker solution of known constituents. Rf value varies from 0.0 – 1.0, so Rf value some time converted into Rf value by multiplying Rf with 100 to obtain range from 0.0 – 100 & used for better characterization.

The spots obtained are visualized either by observing in UV light or by using spray reagents like 1% vannilin-sulfuric acid for steroids & terpenoids, dragendorff reagent for alkaloids etc.

TLC method is rapid & also gives information about the chief constituents of plant drug so enables in assessment of quality of the drug, so TLC is widely used adopted. Furthermore TLC also provide Drug fingerprint, if any adulterants or substituent’s presents they can be detected. So, TLC is used for monitor the identity & purity of the drug.

HPLC:

HPLC assay method is also mentioned in the monographs to estimate the amount of markers present in the drug. In this method standard of known concentration is prepared & injected into HPLC system to get peak area, which is proportional to its concentration. Similarly the peak areas of the sample because of the presence of the active constituents are taken which is equal to that of the quantity of the active constituents present in the drug.

The active constituents are present in the sample which is determined by comparing its peak areas in the both standard & sample, dilutions are also taken into account. For Example -

Gas chromatography is also employed for the analysis of volatile oil & fatty acids.

10. **Refractive Index:**

   It is an important parameter in the evaluation of the volatile oil & fixed oils. Refractive index is defined as the ratio between the velocity of light in the substance or oil or substance under test. It is given by the sine of angle of incidence to the sine of angle of refraction. RI changes if an oil contain some adulterants. It is generally studied at 20°C using Abbe’s refractometer.

11. **Optical Rotation:**

   Many volatile oil & other natural products have the ability to rotate the plane of the polarized light to right or left side, like wise they are called as dextro or levorotatory. This detection of topical & its magnitude is an important criterion for the evaluation of certain drugs. It is studied by using polarimeter at 20°C.

   Along with these above physical constants other physical parameters like melting point, Boiling point, and Freezing point etc are applied for the constituents, which are separated. Any variation in these values or active constituents shows the presence of adulterants.

**Spectroscopic Method:**

After the isolation, purification of the active constituents the chemical structure of which can be determined by using spectroscopic method. The isolated material is also compared with that of standard by using this method. If any adulterant or substituent’s present they will give the additional peaks or alter the λ max.

**Chemical evaluation:**

Just like physical evaluation, active constituents present in the drug can be determined by chemical evaluation E.g., Fixed oil & fats can be determined by acid value, iodine value, saponification value, acetyl value etc. Volatile oils by ester value, acetyl value, Balmas by ester value, acetyl value, saponification value.

In case of drug containing organic acid, non-aqueous titration method is used for the analysis. Gravimetric analysis where we are going to determine the weight of substance. The substance is converted into highly colored complex or chelate, which is analyzed by colorimetric method.
But easier & faster way of the drug is by Phytochemical screening in which simple chemical tests are used for the identification of them.

**Biological evaluation:**

Most of the crude can be assayed by using chemical method but in some cases these methods are not help in the determination of biological efficiency or in the estimation of potency of crude drugs. In such cases biological efficacy or in the estimation of potency of crude drugs. In such cases biological methods are adopted for the evaluation of the activity. E.g. Cardio tonic activity if Digitalis, antidiabetic activity of karela, antioxidant activity of amla etc. Different animals or isolated tissues are used for these purposes. E.g., hypoglycemic activity of karela, fenugreek, and gymnema Sylvester is evaluated in rabbits, rats. Mydriatic action of atropine in rabbits. Anticoagulant activity of heparin in sheep etc.

Isolated tissues like, rectus abdominus muscle for cholinergic action, mast cell degranulation as anti-allergic model, guinea pig ileum for histamine.
1.4: NEED AND OBJECTIVE FOR THE STUDY:

Nature has been a boon to mankind; not only it nourishes us with food but also gives beneficial effect in the treatment or cure the of various types of diseases, i.e the medicinal plants. Thus generally the plants are most useful in the treatments of diseases or many ailments from tribe to tribe in various geographical locations.

The efficacy of the drugs along with its safety factor has made it to be widely use by traditional practisneror physicians now a days. In rural areas the the herbal drugs are most commonly used for treatment of different diseases because they have not mony to purches the synthetic drug. Then, they are used the crude drug which are available in naturally his area or concern to vaidhya. Now a days the synthetic drugs are coming up fast in the market, but these medicines are not suitable for various diseases like cardiovascular disorder, asthma, liver disorder, git disorders etc.

The largest organ in the body is liver and it is most complicated organ of the body than other. The main function of liver is that the metabolism of food stuff and drugs. All the biochemical reactions are occurs in liver. It also maintaine the internal environment of the body. There are various enzymes are present in the liver which catalyzes the biological reactions. The endogenous and exogenous has been continuous involvement, which is sucesrtible for toxic injuries caused by disturb body metabolism. Contineusaly administration of drugs like paracetamol, tetracycline, oral contraceptives, anti-tubercular drugs for hormonal origin, chemicals used which are for the food preservatives and threatening the integrity of the liver [71].

There are available few remedies are identified for the prevalent liver disorders among human being. Presently such type of drugs has not developed in the modern system of medicine which help for the stimulates or cure the liver function, and which are protect from hepatotoxic agents and are help for the reproduced of hepatic cells in liver.

Now, a days the only allopathic drugs are available for treatment of liver disorders like corticosteroids and immuno-suppressive agents. But these agents are used continuously there will be shows some side effects. So, need to developed new or novel drugs which are the treate against damages of liver [64].
According to the review of literature mostly the drugs are obtained from plants which are used to the treatment of liver disorder diseases. Therefore, I have chose the three plants drug for evaluation of hepatoprotective activity.

The treatment of the toxicity of liver in rats is by using the plant extracts like Steroids, Flavonoids, glycoside etc.[44].

The survey of literature reveals that the roots, leaves and fruits of the medicinal plant such as Mangifera indica L., Ricinus communis L. & Caesalpinia bonduc L. are generally utilized for the treatment of number of diseases like diuretic, anthelmintic, antibacterial and brain tonic and keeping off infectious disease. The leaves are used in convulsion, liver disorder, inflammation and has shown to have property of antioxidant [100,5].

**OBJECTIVE OF STUDY:**

- Extensive review of literature of the plant *Ricinus communis* L, *Caesalpinia bonduc* L.& *Mangifera indica* L.

- Preparation of Herbarium of the plant *Caesalpinia bonduc* L., *Ricinus communis* L.& *Mangifera indica* L.

- Collection of selected plants materials of *Caesalpinia bonduc* L., *Ricinus communis* L & *Mangifera indica* L.

- Extraction of leaves of *Caesalpinia bonduc* L., *Mangifera indica* L. & *Ricinus communis* L by using polar and non polar solvents.

- Dring of extracts of *Caesalpinia bonduc* L., *Mangifera indica* L. & *Ricinus communis* L.

- Standardisation of plant of *Caesalpinia bonduc* L., *Mangifera indica* L. & *Ricinus communis* L.

- Phytochemical studies of the plant *Caesalpinia bonduc* L., *Mangifera indica* L. & *Ricinus communis* L.

- Identification of various extracts of Mangifera indica L., Ricinus communis L.& Caesalpinia bonduc L.using chromatographic technique (Thin layer chromatography and HPTLC )
➢ Preparation of herbal formulation of leaves of Mangifera indica L., Ricinus communis L. & Caesalpinia bonduc L.

➢ Acute toxicity of Herbal Formulation. Activity of various extracts on Paracetamol induced hepatotoxicity using different doses of Herbal Formulation.

➢ Histopathological studies of different dose of Herbal Formulation.