CHAPTER I
INTRODUCTION

Traditional medicaments, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet. It is rather difficult to date back the origin of these medicaments as a means of therapy. The informations gathered by the ancestors have been transferred through generations and have become established as systems of traditional medicine in different countries of the world.[1]

Inspite of overwhelming influence of modern medicine and tremendous advances made in the production of synthetic drugs, traditional medicaments, designated now-a-days as herbal drugs in different places in literature, have retained their place in therapy. Their effectiveness, low cost and comparative freedom from serious toxic effects, make these medicaments not only popular but also an acceptable mode of treating diseases even in modern times. Procurement and usage of such herbal drugs traditionally to alleviate human sufferings are perhaps as old as human civilisation.

India, a veritable emporium of plants, occupies the topmost place among the leading users of herbal medicines.[2] A vast majority of Indian population is still treated by the well described traditional systems of medicine, like, Ayurveda, Unani and Siddha. A lot many folklore medicines with a long recorded history on the usage of plants for various purposes are practised in this country. Although many

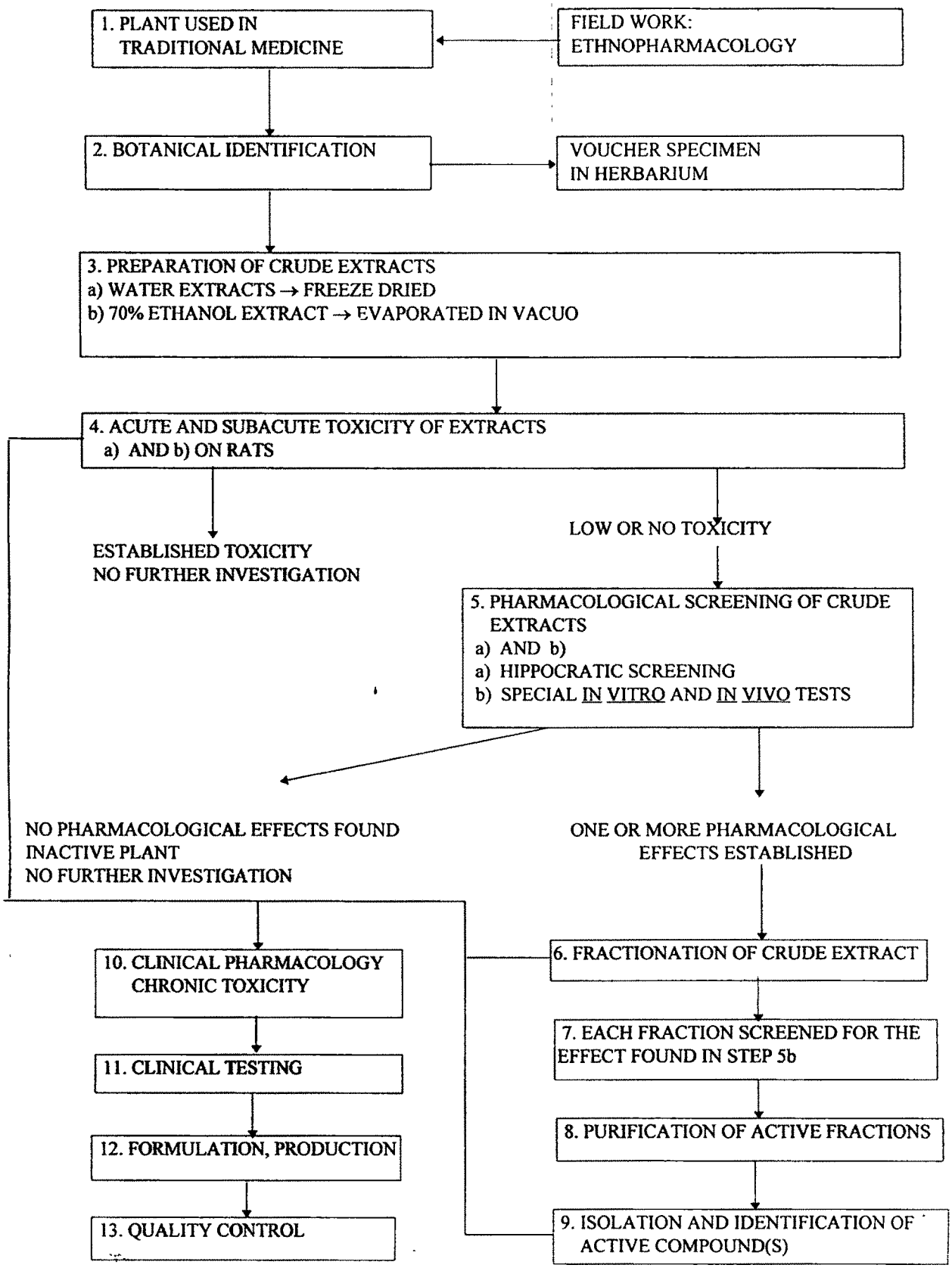

FIG. 1: " CONVEYER - BELT" PROCEDURE FOR DEVELOPMENT OF CRUDE DRUG RESEARCH

developing countries spend a large portion of their total health budget on discoveries and utilisation of drugs, they still depend on herbal remedies as, many items of modern medicine are beyond the reach of three quarter of the third world's population and for them, the utilisation of herbal drugs becomes a necessity.[3] Only a fraction of world's plant population yielding enormous benefits to mankind have so far been studied for their hidden resources of medicinal value. The utilisation of modern scientific methods in exploring possibilities of obtaining better medicaments from the traditional systems has, therefore, become an important task in herbal medicine research.

Crude drug research can be very satisfying and at the same time very frustrating exercise as from the demonstration of some sort of activity to the acceptance of compound in clinical practice is a long road with many pitfalls.[4] There are three main approaches for plant drug research, viz. 1) Classification of plants depending upon their constituents, 2) Random testing of plants for particular biological activity, 3) Selection of plants, based on their usage in traditional or folk medicine i.e. conveyor belt procedure.[5] (Fig.1)

These approaches do have certain advantages. The last one, however, appears to be more logical and widely practised also. It is now a well accepted fact that in future, drug

research on traditional systems of medicine shall have to play a major role by screening not only the plant extracts but also their isolated compounds thoroughly. Since it is still a matter of doubt whether the isolated component only is responsible for exhibiting the same spectrum of activity as that of the drug as a whole, it also becomes an important task to ascertain whether the activity is retained or destroyed during the process of isolation of component.

Although there are about 700 plant products used in various polyherbal formulations, only a few could retain a place in modern medicine due to lack of accurate methods of standardisation and evaluation of therapeutic efficacy. The validation of traditional claims made for these medicaments becomes a very difficult task due to non-availability of suitable experimental methods. There has been an unending quest aroused in the field of herbal product development adopting similar parameters as used in developing modern drug products like composition, formulation development, stability studies, standard methods of manufacturing, quality assurance, and in process controls.

The use of herbal medicine, however, can be made relevant and popular after evaluating these within the frame work of protocols used for modern medicine for their quality and efficacy, thereby bringing the necessary confidence in both the physician and the patient.[6]

The modern system of medicine still lack in providing suitable medicament for a large number of adverse conditions, inspite of tremendous advances made in discovery of new compounds. A few of these diseases can be mentioned like, hepatic disorders, viral infections, AIDS, rheumatic diseases,[7,8] etc. The available therapeutic agents only bring about symptomatic relief without any influence on the curative process, thus, causing the risks of relapses and danger of untoward effects. A large number of population suffer, due to various reasons, from hepatic diseases and also inflammatory conditions of known and unknown origin. The development of anti-inflammatory and hepatoprotective/ antihepatotoxic drugs being a major thrust area, has drawn attention of majority of workers in the field of natural product research. The survey of literature mentions a large number of plant drugs used as traditional medicaments with a claim as effective agents against these disorders. Majority of these traditional medicaments have not been studied and evaluated on modern lines, thus providing a scope and hope of finding out apt medicaments when subjected to a systematic study using reported methods for evaluation of various activities.

1.1 ANTI-INFLAMMATORY ACTIVITY (AIA)

Inflamation, a defensive reaction to injury with

classical signs of warmth, reddening, pain, swelling and loss of function, is of acute or chronic type.[9] The important aspects that render inflammation easy to evaluate are the measurement of erythemea, oedema, formation of granulation tissue, changes in morphological parameters of adrenals and spleen, biochemical parameters such as serum proteins especially -2-macroglobulin levels,[10] serum acid phosphatase (ACP) and serum transaninase levels viz. serum glutamate oxaloacetate transaminase (SGOT) or aspartate transaminase (AST) and serum glutamate pyruvate transaminase (SGPT) or alanine transaminase (ALT), pain threshold, extravasation of plasma-bound dye marker (Evans blue ^{131}I) and local skin temperatures, etc. The anti-inflammatory activity (AIA) of any compound, therefore, can be evaluated by its ability to reduce one or more of these phenomena in an artificially induced inflammation or arthritis in experimental animals.

1.1.1 Experimental Models for Evaluation of Anti-Inflammatory Activity[11]

The various models available for testing anti-inflammatory activity with reasonable accuracy, minimum time and test compound consumption are described below:

i. Acute Models of Inflammation

(a) Carrageenan induced oedema model: Acute hind paw oedema is induced either in mice or in rats by injecting 0.025 ml or

0.1 ml of 1% w/v carrgeenan which reaches a peak level at 3-5 hrs of carrageen injection. Although oedema can be induced by many other phlogestic agents like dextran, formaldehyde, 5-hydroxytryptamine, histamine bradykinin and prostaglandin E_1 etc., for routine screening, acute carrageenan induced oedema test is employed.

(b) U. V. light induced erythema model: Exposure to U.V. radiation also induce acute erythema which is used as model for A.I.A testing.

ii. Chronic Models of Inflammation

(a) Cotton pellet test: Chronic inflammation is induced by the implantation of sterile cotton pellets (50 mg±1 mg) on the back or axilla of the rats asceptically. The peak effect is reached within 7 days.

(b) Granuloma pouch test: Pouch on the back of the rat is produced by injecting 20 ml of air and 1.0 ml of 1% croton oil in olive oil or 0.5 ml of turpentine oil in the subcutaneous tissues in between the shoulder blades. The effect is seen after 7 days.

(c) Formaldehyde induced arthritis: Arthritis is induced by injecting 0.1 ml of 2.0% formaldehyde solution into the subplantar region of one of the hind paws of rat on the first and third day of the 10 days experiment.

(d) Adjuvant induced athritis: Chronic arthritis in rats is induced by injection of 0.5 mg of killed Mycobacterium tuberculosis (Difco) suspended in 0.1 ml of liquid paraffin into one of the hind paws. The effect is observed till 40 days of irritant injection.

1.1.2 Plants with Anti-Inflammatory ACtivity (AIA)

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind and affects a large population of the world, no substantial progress has been made in achieving a permanent cure.[12] The greatest disadvantage of the presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation of treatment. The search for screening and development of drugs for their AIA is therefore, an unending problem and there is a hope of finding out anti-rheumatic drugs from indigenous plants.

The literature survey reveals that the plant species of 96 genera belonging to 56 families have exhibited AIA. Some of the plant sources used in traditional systems of medicine with pharmacologically/therapeutically proven anti-inflammatory and anti-rheumatic claims;[13] are mentioned here like Curcuma longa (Turmeric; Zingiberaceae); Colchicum

autumnale (Colchicum; Liliaceae); Hedychium spicatum (Kapur kachri; Zingiberaceae); Glycyrrhiza glabra (Liquorice; Leguminosae); Boerhaavia diffusa (Punarnava; Nyctaginaceae); Azadirachta indica (Neem; Meliaceae); Ochrocarpos longifolius (Nagkesar; Guttiferae); Randia dumetorum (Mainphal; Rubiaceae); Scutellaria baicalensis (Labiatae), Pluchea lanceolata (Rasna, Compositae), Vitex negundo (Nirgundi; Verbenaceae. Aconitum napellus (Aconite; Ranunculaceae); Commiphora mukul or Balsamodendron mukul (Guggulu; Burseraceae); Alpinia officinarum (Rasna; Zingiberaceae); Boswellia serrata (Salaiguggal; Burseraceae).

The following are some of the natural components reported to possess anti-inflammatory and anti-rheumatic activities, viz., colchicine; glycyrrhizin and glycyrrhetinic acid, curcumin, aconitine, guggulosterone and boswelic acid, etc.

Since these, happen to be long term therapeutic agents, are liable to exhibit some activity on liver also. Therefore, it is an obligatory to assess the effects of anti-inflammatory agents in general on liver also.

1.2 ANTI-HEPATOTOXIC ACTIVITY (AHA)

1.2.1 Liver Disorders

Liver, the largest and the most versatile key organ for

metabolism and excretion, plays an important role in the maintenance of body's internal environment through its multiple and diverse functions. It is continually exposed to a variety of xenobiotics and therapeutic agents due to inadequately controlled environmental pollution and expanding therapeutic uses of potent drugs. Thus, the disorders associated with this organ are numerous and varied. Although a strict delineation of various hepatic disorders is not yet possible, from didactic point of view, these may be classified as acute or chronic hepatitis (inflammatory diseases), hepatosis (non-inflammatory disorders) and liver cirrhosis (degenerative disorder resulting in fibrosis)[14]. The following are some of the liver diseases that are commonly observed as

i. Necrosis

ii. Cirrhosis

iii. Hepatitis may be of viral, toxic or deficiency type

iv. Hepatic Failure

v. Chemical/Drug induced Hepatotoxicity: Generally may be manifested as cholestatic jaundice, hepatocellular or hepatitic jaundice and carcinogenesis.

vi. Liver disorders due to impaired metabolic functions. Generally the disorders associated with fat (liposis) and bilirubin (jaundice) metabolisms are very commonly seen.

(a) Disorders associated with fat metabolism: Fatty Liver.

(b) Disorders associated with bilirubin metabolism: Jaundice

or Icterus which may be of different types based upon mechanisms of action and aetiology.

1. Haemolytic/Prehepatic Jaundice
2. Obstructive/Post-hepatic/Cholestatic Jaundice
3. Hepatogenous Jaundice
4. Hereditary Jaundice: Gilbert's syndrome or Familial hyper bilirubinaemia, Dubin-Johnson syndome and Crigler-Najjar syndrome, etc. are some of the hereditory jaundice types usually observed.[15]

1.2.2 Evaluation of Hepatoactive Medicaments:

i. Screening Methods for Hepatoactivity

Until recently, it had been accepted almost as dogma that there was not and could not be any screening method for standardization and evaluation of hepatoactive drugs since most of the available methods do not simulate the clinical hepatic diseased conditions. Therefore, evaluation of any compound with hepatoactive claims in a single model does not suffice the purpose and needs to be based on multimodels, which are in great demand today.

A review of literature reveals that several chemical substances and drugs having specific actions on liver are used as hepatotoxins in experimental animals to simulate ideal diseased conditions.

Hepatotoxins may be grouped into direct or indirect types depending upon their intrinsic capability, host susceptibility and circumstances of exposure. Generally, as direct toxin injures many tissues including liver (e.g. CCl_4), an indirect toxin affects a particular metabolic pathway of the liver (e.g. galactosamine). Thus, the hepatotoxins affect the liver in a number of ways as:

1. Interference with hepatic bilirubin uptake, conjugation, and excretion e.g. rifampicin.
2. Dose and time-dependent reactions
   a) Acute toxic hepatitis, eg. paracetamol
   b) Fatty liver, e.g. tetracycline
3. Dose-independent reactions
   a) Diffuse Hepatocellular Damage, eg isoniazid
   b) Cholestatic Hepatitis,e.g. chlorpromazine
   c) Granulomatous infiltration, e.g. phenytoin, Chlorpropamide.

Since different hepatotoxins induce hepatotoxicity by different mechanisms of action, there are so many methods are made available for the pharmacological screening, standardization and evaluation of hepatoactive agents, including *in vitro*, *ex vivo* and *in vivo* methods.[16] All these methods are used to study the protective or prophylactic, curative or therapeutic or antihepatotoxic effects of any hepatoactive compound under test. In order to test for hepatoprotective or prophylactic activity, the test substance

and the hepatotoxin are administered simultaneously whereas in case of antihepatotoxic or curative or therapeutic activity, the test substance is generally administered after induction of hepatotoxicity.

(a) in vitro Methods: In these methods, hepatocytes are generally isolated by using in-situ, two step recirculating collaginase perfusion technique. These are then seeded in small containers and exposed to test samples and toxins. After a specified time period, the degree of toxicity or protection is assessed by viability tests and enzyme levels such as SGOT and SGPT.

In vitro models employing primary cultured hepatocytes using CCl_4, galactosamine, ethanol, phalloidin and paracetamol etc. as hepatotoxins have been devised. These have a number of advantages over in vivo methods such as their ability to dispose numerous samples at a time, at a low cost with a small sample size, little variation and reproductibility of results. The major disadvantage is that sometimes it may not reflect the events which occur in animals.

(b) Ex vivo methods: In this, after completion of a preselected in vivo test protocol, hepatocytes are isolated and the percentage of viable cells and biochemical parameters are determained as liver function tests. These methods are

somewhat better correlated to clinical models than either in vitro or in vivo methods alone.

(c) In vivo methods These are of two types as described.

1) Based on Serum Parameters: In vivo methods are used not only to study the nature of the given compound but also to study the mechanisms of action of the toxicant. Hepatotoxicity is produced in experimental animals by the administration of a known dose of a hepatotoxin like CCl_4, D-galactosamine, thioacetamide, alcohol and paracetamol etc., which produce marked and measurable effects, the magnitude of which can be measured by carrying out various liver function tests, viz., morphological, metabolic or functional, biochemical and histopathological determinations. Although it is a very convenient laboratory method, reproductibility of results is rather poor.

2. Based on Bile Parameters: The compounds having hepatoactive claims are also evaluated in general for their choleretic or anti-cholestatic a activity in order to know whether the liver disorder is due to an abnormality of bilirubin metabolism or not. Choleretics are those agents which increase the output of the bile by stimulating the liver whereas anticholestatics are those which correct the retention and accumulation of bile due to intrinsic or extrinsic factors in the liver. These

activities are evaluated by studying bile flow contents in conscious and anaesthetized animals for 5 hours.

1.2.3 Experimental Models for Anti-hepatotoxic Screening

Several chemical reagents and drugs which induce liposis, necrosis, cirrhosis, carcinogenesis and hepatobiliary dysfunctions in experimental animals are classified as hepatotoxins. The most important ones used are carbon tetrachloride (CCl_4), thioacetamide (TAA), D-galctosamine (GalN), paracetamol (Pcl), Chloroform ($CHCl_3$), ethyl alcohol (EtOH), and pyridine. The following are some of the experimental rat models employing these hepatotoxins:

i. CCl_4 Models

A number of CCl_4 models are devised depending upon its dosage through different routes of administration viz.

(a) Acute hepatic damage: Acute liver damage, characterised by ischemia, hydropic degeneration and central necrosis, is caused by oral or subcutaneous administration of CCl_4 (1.25 ml/kg). The biochemical parameters elevated are found to be maximum after 24 hrs of CCl_4 administration.[17]

(b) Chronic, reversible hepatic damage: Administration of CCl_4 (1 ml/kg,s.c.) twice weekly for 8 weeks produces chronic, reversible liver damage.[18]

(c) Chronic, irreversible hepatic damage: Administration of CCl_4 (1 ml/kg,s.c.) twice weekly, for 12 weeks simulates chronic, irreversible liver damage.[19]

ii. Thioacetamide Model

Thioacetamide (100 mg/kg,s.c.) induces acute hepatic damage after 48 hrs of administration by causing sinusoidal congestion and hydropic swelling with increased mitosis.[20]

iii. D-Galactosamine Model

D-galactosamine (800 mg/kg,i.p.) induces acute hepatotoxicity after 48 hrs of administration, with diffused necrosis and steatosis[21]

iv. Acetaminophen (Paracetamol) Model

Paracetamol induces acute hepatotoxicity depending upon its dosage through different routes of administration, such as

(1) Paracetamol (800 mg/kg,i.p.) induces centrilobular necrosis without steatosis.[22]

(2) Paracetamol (3.0 g/kg,p.o.) simulates acute hepatic damage.[23]

v. Chloroform Model

It produces hepatotoxicity with extensive central necrosis, fatty metamorphosis, hepatic cell degeneration and necrosis either by inhalation (for 1 hr in atmosphere) or by

subcutaneous administration (0.4-1.5 ml/kg).[24]

vi Ethyl Alcohol Model

Ethanol induces liposis to a different degree depending upon its dose, route and period of administration, which is hastened by very low doses of other toxicants such as:

(a) A single dose of ethanol (1.0 ml/kg,s.c.) induces fatty degeneration.[25]

(b) Administration of 40% (v/v) ethanol (2 ml/100 g/day, p.o) for 21 days produces fatty liver, which is hastened by administration of CCl_4 (0.1 ml/kg, s.c. of CCl_4 in oilve oil in 1:1 ratio) on 20th day.[26]

(c) Administration of country made liquor (3 ml/100 g/day, p.o.) for 21 days produces liposis.[27]

1.2.4 Experimental Models for Choleretic And Cholestatic Activity[28]

i. Paracetamol-Induced Cholestasis

A single oral dose of acetaminophen (1.5 g/kg) produces cholestasis with a marked hepatic damage after 48 hrs of its administration.

ii. Ethynylestradiol (EE)-Induced Cholestasis

Administration of ethynylestradiol (5 mg/kg,s.c.) for 3

days on the 5th, 6th and 7th days of treatment, increases bile flow by 24 hrs after its last dose.

1.2.5 Mechanism of Action of Some Selected Hepatotoxins

i. Carbon Tetrachloride (CCl_4) [29,30]

The hepatotoxicity of CCl_4 is due to the metabolic formation of the highly reactive trichloromethyl free radical which attacks the polyunsaturated fatty acids of the membrane of the endoplasmic reticulum and initiates a chain reaction. It is enhanced by induction of hepatic microsomal enzyme systems and vice versa or by antioxidants which mop up the free radicals. The first cells to be damaged are those in the centrilobular region where microsomal enzyme activity is the greatest. The initial damage produced is highly localised in the endoplasmic reticulum which results in loss of cytochrome P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver, a characteristic of CCl_4 poisoning. If the damage is severe, it leads to disturbances in the water and electrolyte balance of hepatocytes leading to an abnormal increase in liver enzymes in plasma, thereby impairing mitochondrial function, followed by hepatocellular necrosis.

ii. Acetaminophen [31]

Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses. Overdoses, however, taken with

suicidal intent, produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion, but when the conjugating enzymes are saturated the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative by Cytochrome P_{450} enzyme. The hydroxylamine derivative, a reactive electrophilic agent, reacts non-enzymatically with glutathione and detoxifies. When the hepatic reserves of glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes.

iii. Thioacetamide[32]

Thioacetamide, a substitute for H_2S with less toxicity and obnoxious smell, on repeated exposure produces cirrhosis by inhibiting the respiratory metabolism of the liver due to the uncontrolled entry of Ca^{+2} ions into hepatocytes, resulting in inhibition of oxidative phosphorylation. Early metabolic disturbances increase the RNA and protein content of the nuclear fraction of hepatocytes leading to varying graded liver damage including nodular cirrhosis, liver cell proliferation, production of pseudolobules and parenchymal cell necrosis. The serum levels of glutamic dehydrogenase are also found to increase, indicative of mitochondrial injury, which plays an important role in thioacetamide induced hepatotoxicity.

iv. D-galactosamine[33]

Generally, it produces reproducible liver cell injuries. The galactosamine hepatitic livers have great resemblance with livers in human viral hepatitis in its morphological and functional features.[34] During its metabolism in the liver, the level of several uracil nucleotides (UTP, UDP-glucose, and UDP-galactose) is depleted, resulting in the inhibition of RNA synthesis and disturbance of the biosynthesis of glycoproteins, leading to deterioration of the cell membranes. Morphologically it produces both diffused necrosis and steatosis.

v. Rifampicin[35]

All the anti-tubercular drugs are liable to produce liver damage with a broad spectrum of liver dysfunction, ranging from slight elevations of transaminases to severe hepatocellular necrosis.

Rifampicin, a broad spectrum antibiotic, is the most commonly and widely used antitubercular drug, particularly in combination with isonicotinic acid hydrazide (INH) or other similar agents. Both rifampicin and INH are known to be hepatotoxic, when administered separately and continued particularly in combination with INH. Although rifampicin does not appear to accumulate in hepatic dysfunction, it should be used cautiously because of its hepatotoxic nature.

It is largely metabolised to desacetyl rifampicin, which undergoes entero-hepatic circulation. It is distributed throughout the body, and about 85% of it gets bound to serum proteins. Since it is largely metabolised in liver, its serum concentration rises in liver diseases with fatal liver damage and acute hepatic failure. It reduces drug metabolizing enzymes in liver and thereby impairs other drugs effectiveness. Since it actively and specifically binds to RNA polymerases, it inhibits the synthesis of all forms of RNA. Thus, by inhibiting nucleic acid and protein synthesis, it induces fatty liver and finally cirrhosis.[36]

1.2.6 Assessment of Hepatic Function

The detection of toxic changes in the liver during the early stages of therapy of moderate deficiencies is a challenge, because of its difficulties in finding tests solely dependent on changes within the liver and poor correlations between the clinical, histological and biochemical findings in liver diseases.

Investigations on liver functions are generally confined to the detection of constituents of blood or urine, whose excretion, catabolism or metabolism involves the liver or the tests involving stress by over loading the liver with an agent administered in excess.[37] Recent developments in the diagnosis of disorders of liver and biliary system,

specially due to drugs and chemicals, have evolved a range of sensitive and specific tests for the identification of the type of injury, status, and working of liver. The following are some of the parameters commonly employed for the evaluation of liver injury.

i. Functional/Metabolic Assessment

These evaluations, generally based on various functions of the liver, are further catagorized as clearance, non-invasive clearance and immunological tests.

(a) Hepatic clearance tests[38] The clearance capacity or the ability of the liver to remove certain indicator substances from the blood stream and to secrete them into the bile provides an useful diagnostic test of liver functions especially in altered hepatic blood flow, hepatocyte dysfunction or abnormalities of the biliary passage. The test substances which have been widely used for this purpose are dyes and barbiturates.

1) Dyes : The dyes, which are widely used for this purpose, bromosulphthalein (BSP), indocyanine green (ICG) and rose bengal, are injected intravenously as sodium salts. These anions are strongly bound to plasma albumin, so that little or none appears in glomerular filtrate. However, these are rapidly and completely cleared from blood stream by uptake into liver and then secreted into bile.

BSP (5 mg/kg,i.v.) is completely cleared fromblood stream within 45 min by the normal liver. However, this process is delayed in case of liver dysfunction. ICG is now preferred because of its greater reliability and less toxicity compared to BSP.

2) Drugs: Barbiturates i.e. pentobarbitone or hexobarbitone induced sleeping time is a commonly used parameter for the assessment of liver function. These are administered (30 mg/kg) intraperitonially and a mean sleeping time of 9 min in case of mice and 23 min in case of rats are selected, since it prolongs significantly in liver dysfunction.

b) Non-invasive hepatic clearance tests: Non-invasive clearance of bile acids endogenous metabolites and aminopyrine are taken as methods for the assessment of liver function, because of the invasiveness and untoward reactions of dye clearance tests.

1) Serum Bile Acids: The level of bile acids in peripheral blood provides an index to the function of hepatocytes, which take these from the portal vein and excrete into bile. Their concentration in portal vein plasma in turn depends on the amount presented to and transported by the distal ileum. Since the peripheral plasma bile acid levels rises with hepatic dysfunction, which may be

evident with an increase in fasting bile acids or continued increase in bile acids following ingestion of a meal due to an increased load.[39]

2) Aminopyrine clearance:[40] Aminopyrine is rapidly absorbed and evenly distributed in total body water and mainly metabolised in liver, primarily through N-demethylation. Therefore, the rate of hepatic metabolism of dimethyl amino antipyrine (aminopyrine) may also be used as a parameter to assess liver function. It's clearance may be evaluated by using a breath test.

3) Serum transaminases / aminotransferases: The most commonly used enzymes for assessment of liver function are serum transaminases or aminotransferases, viz aspartate aminotransferase (AST) or serum glutamic oxalacetic transaminase (SGOT) and alanine aminotransferase (ALT) or serum glutamic pyruvic transaminase (SGPT), which can be estimated by spectrophotometric and automated techniques. These are normally present inside the liver cells and their blood levels rise in cell damage. Elevation of serum transaminase levels even with minor cell damage makes these determinations useful especially in the early detection and monitoring of drug or chemical induced hepatotoxicities. However, the lack of specificity is a serious drawback. Chemical induced hepatitis, such as

CCl_4, may produce very highly elevated levels of these enzymes.[41]

4) Serum Phosphatases: Serum alkaline phosphatase (ALKP) widely distributed in various organs of the body, is useful in detecting liver or biliary tract diseases. Therefore, hepatotoxicities which result in disturbances in the transport function of hepatocytes or of biliary tree elevate serum alkaline phosphatase levels.[42]

5) Serum 5'-Nucleotidase: Estimation of serum 5'-Nucleotidase plays an important role in distinguishing between hepatic and nonhepatic causes of elevated serum alkaline phosphatase levels. Its distribution in hepatocytes is found to be similar to that of alkaline phosphatase and it plays an important physiological role in membrane mediation.[43]

6) Serum-γ-Glutamyl Transferase: It is a more sensitive indicator compared to aminotrasnferases in case of chemical drug and alcohol induced hepatotoxicities and alkaline phosphatase, in the detection of biliary obstructions. Since its increase following drug intoxication is due to enzyme induction, it can be used as a marker in addition to other tests.[44]

7) Glutamic Dehydrogenase: It is completely a mitochondrial

enzyme. Therefore, can be used for monitoring and studying the mechanisms of action of drug or chemical induced hepatotoxicities. The mechanism of action of any substance which induces hepatotoxicity by attacking mitochondria first, can be studied easily by estimating glutamic dehydrogenase levels early,[45] e.g., thioacetamide.

8) Serum Bilirubin: Serum bilirubin, a diagnostic feature in detection of liver damages, is determined by reaction with diazotized sulphanilic acid. Generally, normal serum contains unconjugated bilirubin, whereas the presence of conjugated bilirudin in serum indicates the liver or parenchyma or bile duct dysfunction.[46]

9) Serum Albumin: Serum albumin, an important parameter in the detection of the severity of cellular dysfunction in liver diseases, is commonly determined by using bye binding and immunologic techniques. It is of little value in differential diagnosis or recognition of lessions due to brug or chemical induced hepatotoxicity. Abnormal distribution, increased plasma volume and possible abnormal degradation may also contribute to hypoalbuminemia in liver disease.[47]

10) Prothrombin Time: The one-stage prothrombin time is a simple method for the evaluation of hepatotoxicity.

Although it is a relatively insensitive indicator of liver damage, it is of little value in differential diagnosis. Hypoprothrombinemia is the first evidence of liver damage due to drugs or chemicals. Prothrombin time, a specific measure of plasma prothrombin (factor II), is also depends on factors V, VII, X, and fibrinogen. A reduction in any of these factors also prolongs the prothrombin time. Therefore, is a sensitive method for determining the marked depresion of vitamin K dependent coagulant factor levels prodced by anticogulants.[48]

11) Urinary Ascorbic Acid: Ascorbic acid, formed as a metabolic of glucose and galactose by the enzymes UDP glucose dehydrogenase and UDP glucuronide transferase in rat liver microsomes via the glucuronic acid path way, is excreted in the urine.[49] Its formation and excretion is altered by several drugs and substances that affect the drug metabolising enzyme systems. Thus, alteration is urinary ascorbic and excretion appears to be reflecting ascorbic acid levels in liver. Hence, the reduction in urinary ascorbic acid excretion can be used as an index for CCl_4 induced hepetotoxicity. It is determined by using Roe and Kuether method.[50]

(c) Immunologic studies: During clinical trials immunologic studies are carried out in patients with suspected liver damage, to differentiate liver damage from that caused by

various infective agents such as hepatitis A or B virus; to detect specific antibodies to various drugs, chemicals or their metabolites, to assess overall immunologic reactivity and to access the patient for antigens originating from liver such as liver specific protein and alcoholic hyaline. Although these studies may not yield positive result every time, still these are of important in liver toxicity studies.[51]

ii. Morphological Assessment

It is essential in demonstrating the presence, nature, diversity and activity of hepatotoxin in drug or chemical induced hepatotoxicity. Generally, the animals intoxicated with hepatotoxins demonstrated significant changes in body weight, liver weight and volumes eg CCl_4. Therefore, the morphological assessment alongwith biochemical assessment is useful in establishing the mechanism of action of a toxicant and the probable effective route of administration of a hepatoactive test substance.[52]

iii. Histological Assessment

For histopathological evaluation, haematoxylin-eosin stained liver sections are usually employed as a diagnostic tool.[53] These thin stained sections are observed for the cleavave occurring due to intoxication and the protective effect of a drug. The commonly used studies under light microscope are degree and type of necrosis or cirrhosis and

the arrangement of cell and blood capillaries, whereas the electron microscopic studies include ultrastructural alterations such as plasma memberane, mitochondria, golgi apparatus, lysosomes, endo plasmic reticulation and peroxisomes. The other histopathological studies including fluorescence microscopy, autoradiography and immunocytology are also the useful techniques in the diagnosis and evaluation of mechanism of toxic effects of a hepatotoxin.

iv. Assessment Through Kinetic Studies

Evaluation of nucleic acid and collagen synthesis is possible by in vitro incorporation of precursors into these moieties. Use of radioisotopes of high specific gravity, which develop autoradiographs within 4-6 hrs, made these studies further simple. Hepatotoxicity with hepatocytes, biliary duct or mesenchymal injury is characterised by significant increases in DNA and collagen synthesis. A regenerative phase of progressive injury exhibits negligible incorporation of precursors. Therefore, monitoring of the efficiency of preventive and therapeutic measures are possible with these studies.[54]

1.2.7 Hepatoactive Medicaments

The literature survey reveals a large number of drugs of plant origin are endowed with hepatoactive claims either directly or indirectly. These are generally classified into

three categories without any strict delineation among them, viz.

1. Antihepatotoxic agents: These generally antagonise the effects of any hepatotoxin causing hepatitis or any liver disorder or disease.
2. Hepatotropic agents: These generally support or promote the healing process of the liver,

   In practice these two activities can not be easily distinguished from each other.
3. Hepatoprotective agents: These generally prevent various types of liver affections prophilactically.

In general, any hepatoprotective agent can act as an antihepatotoxic or hepatotropic agemt, but the vice versa is always not true. The following are some of the plant drugs having hepatoprotective claims.

i. Plant Drugs Used in Liver Disorders

There are about 160 phytoconstituents from 101 plants belonging to 52 families have exhibited antihepatotoxic activity. In India we have over 40 commercial polyherbal formulations available for hepatoprotective activity.[55,56] Some of the crude drugs with proven activity against liver diseases are:

Andrographis paniculata (Acanthaceae); Boerhaavia diffusa (Nyctaginaceae); Bupleurum falcatum (Umbelliferae); Eclipta alba (Asteraceae); Glycyrrhiza glabra (Leguminosae);

Phyllanthus amarus (Euphorbiaceae); Picrorrhiza kurroa (Schrophutariaceae), Schizandra chinenris (Schizandraceae); Silybum marianum (Compositae); Unecaria gambir (Rubiaceae).

Some of the reported constituents with pharmacologically/therapeutically proved claims may be enlisted as: silymarin, glycyrrhizin, (+)-catechin; schizandra lignoids, saikosaponins, andrographolides, curcumin and picroliv etc.[57]

Other than the above mentioned plants, there are lot many appear in the literature having claims of activity against different types of inflammatory diseases including liver disorders. The most commonly used ones worth mentioning are as follows:

Withania somnifera (Solanaceae); Ricinus communis (Euporbiaceae); Butea monosperma (Leguminosae), Allium sativum (Liliaceae); Calotropic procera (Asclepiadaceae), Tinospora cordifolia (Merispermaceae); Piper longum (Piperaceae), Cyperus rotundus (Cyperaceae), Solanum nigrum (Solanaceae), Curculigo orchoids (amaryllidaceae/ Hypoxidaceae), Fumaria indica (Fumariaceae/Papaveraceae), Inula rasemosa (Asteraceae/ compositae), Moringa pterygospema (Moringaceae), Sida spp. (Malvaceae,) Swertia chirata (Gentianaceae) etc.

From the above list of plant drugs it was revealed that a few of these such as rhizomes of Curculigo orchioides Gaertn (Hypoxidaceae), roots of Inula racemosa Hook.f. (Asteraceae), whole plants of Fumaria indica (Hausk) Pugsley (Fumariaceae), and of different species of Sida viz., Sida acuta Burm, Sida cordifolia Linn. and Sida rhombifolia Linn. (Malvaceae) and different parts viz fruits, leaves, stem bark and roots of Moringa pterygosperma Gaertn (Moringaceae) also form as components of various polyherbal formulations, without any systematic evaluation on their endowed claims. These drugs were therefore, chosen for a detailed systematic investigations. A detailed review of literature, therefore, was undertaken on these plant drugs used in traditional systems of medicine and the informations available were compiled under individual heading of each drug.

1.3 LITERATURE SURVEY OF SELECTED PLANT DRUGS

1.3.1 Curculigo orchioides Gaertn.

The rhizomes of Curculigo orchioides Gaertn. (Amaryllidaceae / Hypoxidaceae), commonly known as "Kali Musli" and "Siyah Musli" form an important constituent of several Ayurvedic and Unani drug formulations. Curculigo orchioides Gaertn is a small, perennial, geophilous, Scapigerous monsoon herb, greatly resembling a small young palm. It bears a few small, bright yellow distichous flowers.

The plant is indigenous to India. Musli is a powerful drug of Ayurvedic system, agreeable and bitter in taste, a nourishing tonic which gives strength and destroys all ailments pertaining to the anal region i.e. piles. It suppresses "Vat" and "Pitta", increases vigour and vitality; gives long life. It is pleasing and increases secretions of body fluids and keeps one healthy and robust. It is useful in fever, joint pains, cuts, diseases of nerves, gleet, lumbago, vomiting, dyspepsia, hydrophobia, diabetes and keeps the three "doshas" ("Vayu", "Pitta" and "Kapha") in proper balance. It removes the burning sensation due to hyperacidity, cures diseases of the blood, jaundice, asthma, diarrhoea, gonorrhoea and is a restorative. The crushed rhizome is given in a kind of veneral disease known as "Rukhi". The rhizomes are considered edible and a cooling medicine. The juice of the rhizome mixed with garlic is used as an eye drop to cure blindness and white spots on the eye-ball. Leaves are reported to be used in the treatment of cancer. It is used in phosphatic diathesis and in scleroderma.[58]

The plant yields a powerful uterine stimulant flavone glycoside.[59] The root stalk contains glycosides, sugars (hemicellulose and other polysaccharides), starch, resin, tannin, mucilage, fat and calcium oxalate.[60] The carbohydrates from the tubers are composed of free sugars

like xylose and glucose, mucilage (composed of mannose, glucose glucuronic acid), hemicelluloses, and other polysaccharides.[61] The rhizomes are also reported to contain a sapogenin, an alkaloid lycorine, sterols including -sitosterol[62], phenolic glucosides,[63,64] aliphatic long chain methoxy ketones,[65-67] immunoadjuvants,[68] and cycloartane type triterpene glycosides[69] termed as curculigo saponins which are reported to increase the weight of the thymus.[70]

Kali Musli (C. orchioides) is incorporated in the following marketed formulations available for a variety of disorders:

Ashwabal (Raka Labs., Ahmedabad)

Gypex (Synthochem, Bareilly)

Kupid Fort (Pharma Products Pvt. Ltd., Thanjavur)

Muslipak (Shree Baidyanath Ayurved Bhavan Ltd., Nagpur)

Sensaspur (Vilco Labs Pvt. Ltd., Bombay)

Vigorex (Zandu Pharm Works Ltd., Bombay)

Vinomyn Forte (Myncil Pharmaceutical, Varanasi)

Virilex (TTK Pharma Ltd., Madras)

1.3.2 Fumaria indica Pugsley

The whole plant of Fumaria indica (Hausk.) Pugsley. (Syn. F. vallantii Loisel., Fam: Fumariaceae/Papaveraceae), commonly known as Parpet, Pitpapra, Pitpapda and Shahterah, forms a constituent of many patent Ayurvedic and Unani

preparations and also used as common household medicine.[71]. It is known as Fumitory in European countries.

The plant is a pale green, much branched annual herb with narrow segmented leaves, pink or whitish with purple tipped or leaf opposed raceme type flowers, and with globose, one seeded fruits.[72] It is distributed all over India as a weed and is commonly seen on road sides and hills. It flowers and fruits during the cold season.[73]

The plant is reputed to possess several medicinal properties. It is bitter, slightly acrid and astringent. It is regarded as a diuretic, diaphoretic, aperient, laxative, alterative and anthelmintic. It is used in low fever, to purify blood, dyspepsia and also in scrofulous skin disorders.[74] The seeds are used as a remedy for body pains and the whole plant as fodder. Decoction or an infusion of the herb is recommended in leprosy and syphillis.[75]

The plant is reported to contain alkaloidal principles of different types[76-89] like isoquinoline, benzyl-isoquinoline, spiroisoquinoline, morphinandienone, protopine, tannins, phlobaphenes, sugars with predominant potassium salts, rutosides, quercetin, monoglucosides, phenolic acids like caffeic, chlorogenic and ferulic acids,[90] non-nitrogenous compounds like 19-methyloctacosan-1-ol, C_{27-29} n-alkanes, sterols (β -sitosterol, stigmasterol, campesterol,

4:2:1), and 3-methyloctacosan-1,3-diol[91].

The leaves and stems are found to contain protopine, nonacosanol, tetrahydrocoptisine, norlumidine and sitosterol[92,93]. The seeds contain fixed oil composed of oleic, linoleic, stearic and palmitic acids,[94] dihydrocoptisine, nor, oxy and (±)-8-methoxydihydro sanguinarines, (+)-adlumidine, (±)-bicuculline, fumariline, norceimicine and choline.[95-99] The aerial parts are found to contain 26 isoquinoline alkaloids like fumaramine, corydine and juziphine[100] etc. whereas roots contain protopine, dl-tetrahydrocoptisine, β-sitosterol, octacosanol[101] and norpallidine.[102]

Fumitory alkaloids viz. protopine and fumoficinaline, showed anti-inflammatory and CNS activities,[103] while tetrahydrocoptisine and protopine possessed neuroleptic, smooth muscle relaxant and hydrocholeretic effects.[104,105] Fumaritine is reported to produce sedative, anticonvulsant, and antinociceptive effects.[106]

Pitpapda (F. indica) is incorporated in the following marketed polyherbal formulations available for liver disorders:

Acti-Liv-Forte (Anuja Parmaceuticals, Bombay)

Alarskin (Alarsin Marketing Pvt. Ltd., Bombay)

Amlycure (Aimil Pharmaceuticals (I) Pvt. Ltd., New Delhi)

Amygesic (Aimil Pharmaceuticals (I) Pvt. Ltd., New Delhi)

Gro Liquid (Swastik Formulations Pvt. Ltd., Varanasi)

Hepa-10 (Jupiter Pharmaceuticals, Calcutta)

Heptomyn (Myncil Pharmaceuticals, Varanasi)

Liv 52 (The Himalaya Drug Co., Bangalore)

Livfit (Dabur (I) Ltd., Sahibabad)

Livoerb (Alkem Labs. Ltd. Bombay)

Livomyn (Charak Pharmaceuticals (I) Pvt. Ltd., Umbergaon)

Livosar (Sarvodaya Labs., Bombay)

Livpar (Gufic Ltd., Navsari)

Naturoliv (ACE Labs. Ltd., New Delhi)

Peptoherb (Walter Bushnell Ltd., Bombay)

Prepetone (Swastik Formulations Pvt. Ltd., Varanasi)

Safi (Hamdard (WAKF) Labs., Ghaziabad)

Shilpa Parila (Shilpa Chem., Indore)

Stimuliv (Franco Indian Pharmaceutical Pvt. Ltd., Bombay)

Styplon (The Himalaya Drug Co., Bangalore)

Wormonil (K.K.Shuddha Ayurvedic Pharmacy, Mehmadabad).

1.3.3 *Inula racemosa* Hook.f.

Inula racemosa Hook.f. (Fam: *Asteraceae/ Compositae*), commonly known as Poshkar, is a tall, stout herb with large heads in racemes, leathery leaves and slender, hairless fruits.[107] The roots, commonly known as Pushkarmoola, form an important ingredient of several polyherbal formulations. These are generally grey in colour, 10-15 cm long and upto 2

cm in diameter with a camphoraceous, sharp, hot taste and odour.[108]

The roots are used as a tonic, stomachic, alexiteric, carminative, expectorant, and as an adulterant for the roots of Saussurea lappa. These are good for hemicrania, eruptions, inflammations, ear pain, cough and boils. The drug also dispels the effects of shock, cures pains of the heart, spleen, liver and joints. The root extract showed anti-inflammatory, antispasmodic, analgesic and antipyretic activities without any anabolic effect. The aqueous decoction is reported to lower fasting blood sugar in normal and hyperglycaemic rabbits. The 50% ethanolic extract of the whole plant showed hypoglycaemic effects only in normal rabbits. The essential oil from these roots showed anthelmintic activity against earth worms and tape worms,[109] antibacterial activity against E. coli and anti fungal activity against Fusarium solani.[110] Clinically, the drug was found to improve pulmonary functions, haematological picture and general health. Pretreatment with its powder prevented post exercise ST segment against depression in patients with ischaemic heart disease. The seeds are bitter and aphrodisiac. Generally they strengthen the hair and prevent them from falling.

The powdered root showed the presence of saponins, -sitosterol, octadecanoic acid and D-mannitol.[111,112] The

essential oil from the roots contains alantoids viz., alanto, alloalanto, neoalanto, isoalanto and dihydroiso-alantolactones. The drug also contains inunolides, dihydroinunolides, steroids, terpene:(-)-dammara-20,24, dien-3-B-yl-acetate, aplotaxene and isoalantodienes, telexin, inunal, isoinunal, and oxygenated alantolides such as two epoxyalantolides and one perhydroxy derivative.[113-122] Alanto and isoalantolactones showed in vitro antifungal activities without any significant effects on experimental ring worm infections.

Pushkarmool is incorporated in the following marketed polyherbal formulations available for a variety of disorders:
Aimil cough syrup (Aimil Pharm. (I) Pvt. Ltd., New Delhi)
Amrybion Tablets (Aimil Pharm. (I) Pvt. Ltd., New Delhi)
Kafsin (Yogi Pharmacy, Haridwar)
Kofostal (Nukem Remedies Ltd., Bombay)
Kuftone (Dharmani Drugs Research & Training Inst. Gurgaon)
Kumariasava (Sandoz Ltd, Bombay)
Shilpa Cough (Shilpa Chemi., Indore)
Tusidal Cough (Alidac Genetics & Pharmaceuticals, Ahmedabad)
Vitoherb (Walter Bushnell Ltd., Bombay)

1.3.4 Moringa pterygosperma Gaertn.

Moringa pterygosperma Gaertn. or M. oleifera Lam. or M. adans (Fam: Moringaceae), commonly known as "Drum Stick tree"

and "Horse Raddish tree", since the roots are used as substitute for horse raddish. The plant is a small or medium sized tree grown throughout India. Leaves, flowers, pods and even twigs are cooked as pot-herbs. The immature pod is used in making curry and pickles. All parts of the tree are considered medicinal and used in the treatment of ascites, rheumatism, etc. The plant is ascribed in traditional medicine for various purposes.[123]

The roots are carminative, stomachic, abortifacient, cardiac tonic and also used in paralytic conditions, in intermitent fever, as rubefacient in rheumatism, in spasmodic affections of the bowels, hysteria and flatulence as well as in epilepsy. Root bark is used as fomentation to relieve spasm. Stem bark is considered to be an abortifacient. The fruit is recommended in diseases of liver, and spleen, in tetanus and paralysis.[124] Flowers are stimulant and aphrodisiac.[125] Seed oil is applied externally in rheumatism. Leaves are emetic and their juice, with black pepper is used in headache. The poultice of leaves is used in reducing glandular swellings. The gum is given in dental caries with sesame oil and also for relief of otalgia and is applied with milk in headache. Seeds are used in venereal affections and to relieve the pain of gout and acute rheumatism.

The plant is considered to be useful by tribals in

burns, sores, epilepsy, adenitis, scrofulosa colli, erysipelas, scabies, retention of urine, haematuria, urinary gravel, cholera, dysentery, pneumonia, female sterility, snake bite, scorpion, centipede and spider stings. The seeds are useful in ascites associated with enlarged liver and spleen.

The root bark contains two alkaloids viz., moringine and moringinine, with the total alkaloid content of 0.1%, traces of essential oil, phytosterols, waxes and resins. The roots contain an antibiotic principle, pterygospermin having antifertility activity.[126-130] The aqueous extract of roots was reported to have antifertility activity without any significant changes in histological features in the ovary during early pregnancy in rats.[131,132]

The stem bark revealed the presence of sterols, terpenes, saponins, tannins, mucilage and absence of alkaloids. The hexane extract yielded a triterpenoid, bayrenol. The ethanolic extract of stem yielded 4-hydroxymellein, vanillin, β-sitosterol, β-sitosterone and octacosanoic acid. The leaves are found to be rich in vitamins 'C' and 'A', free amino acids and α- and β-carotene.[133]

Different parts viz., leaf, stem, stem bark, root and fruit are found to be devoid of tanins. The leaves, flowers

and fruits are rich in minerals and vitamins. The seed oil contains oleic, behenic, stearic and palmitic acids.[134-136] The pods also contain mucilage in addition to ascorbic acid oxidase.[137,138] The gum exuded from the tree contains enzymes and bassorin.[139]

Shigru or Sahajna (M. pterygosperma) is incorporated in the following marketed formulations available for a variety of disorders, viz.,

Livospin (Herbals (APS) Pvt. Ltd., Patna)

Kupid Fort (Pharma Products Pvt. Ltd., Thanjavur)

Orthoherb (Walter Bushnell Ltd., Bombay)

Rumalaya (The Himalaya Drug Co., Bangalore)

Septilin (The Himalaya Drug Co., Bangalore)

1.3.5 Sida species

The plants belonging to the genus Sida (Fam: Malvaceae) are commonly known as "Bala".[140] Although there are about 120 species of Sida, only five of them under the name Panchbala are well known. They are:

1. Bala - S. cordifolia Linn.
2. Mahabala - S. rhombifolia var. rhomboidea Roxb.
3. Nagabala - S. spinosa
4. Atibala - S. rhombifolia Linn.
5. Bala Panijivika - S. acuta Burm./S. caprinifolia Linn.

Generally Sida cordifolia Linn., S. rhomboidea Linn. and S. acuta Burm of the Malvaceae family are used under the name "Bala" and are incorporated in a number of traditional polyherbal formulations. Therefore, for the present study only these three species were selected.

i. Sida acuta Burm.

Sida acuta Burm./S. caprinifolia Linn. (Fam: Malvaceae), commonly known as "Bala Panijivika" is an erect perennial shrub with stellate, hairy branches, lanceolate and serrate, 2.5-6.3 cm long leaves, yellow flowers, 5-6 cm diameter black smooth seeds.[141] The drug not only serves as an important medicine but also yields good amount of fibre.

Leaves are considered as demulcent, diuretic and antirheumatic. These are smeared with gingelly oil and applied to suppurate ulcers. The leaf juice is boiled in oil and applied to testicular swellings and in elephantiasis. The juice is also given to relieve chest pain and as an anthelmintic. The leaves are also used for making poultice for sores and frequently to cause abortion. The slimy bruised leaves are put on the hands of midwives to remove dead children from the womb. The liquid obtained by mashing them in water is used as an enema for paralysed children to help them to walk. A decoction of leaves is credited with emollient and tonic properties and is used in the treatment of haemorrhoids and impotence.

Roots are bitter and said to possess astringent, tonic, cooling, stomachic, diaphoretic and antipyretic properties. These are used in nervous and urinary diseases, disorders of blood and bile and in chronic bowel complaints. Fresh root juice is applied to wounds and ulcers and is also used as an electuary and vermifuge. A strong decoction of roots is given in mild cases of debility, rheumatic affections and gonorrhoea.

The plant is also used to dispel colic, as enema, sedative and as a remedy for conjunctivitis. The water soluble fraction of the alcoholic extract of these plants exerted spasmodic action on smooth muscles of ileum, trachea, uterus and heart similar to that of acetyl choline in experimental animals.

Four alkaloids have been reported to occur in the aerial parts of the plant and three in the roots.[142] Seeds contain 0.26% and roots 0.07% of alkaloids. The aerial parts also contain cryptolepin, hydrocarbons, normal and branched chain alkanes, pristane, phytane, hentriacontane, nonacosane, and phytosterols such as campesterol, stigmasterol, B-sitosterol and stigmast-7-enol whereas cholesterol and ergosterol are absent.[143-145] Stems contain β-sitosterol.[146] The seed oil contains cyclopropenoid fatty acids such as sterculic and malvalic acids.[146-148]. The roots contain -amirin, starch,

ecdysterone[149], oxalic acid, an unknown compound X, m.p. 222°-4° and a polysaccharide yielding an acidic xylan.[150] Medicinal applications of these plants have been related to their alkaloidal constituents.[151] Cryptolepine, the major alkaloid of S. acuta showed antimicrobial activity against P. vulgaris. The antibacterial activity of alkanes, alkanols and sterols of these plants were also studied.[152]

ii. Sida cordifolia Linn.

Sida cordifolia Linn. /S. herbacea Micans. (Fam: Malvaceae), commonly known as "Bala"/"Kungyi", enters into the composition of several herbal medicines of Ksirabala Taila, Balaguducyadi Taila, etc. as an antirheumatic drug.[153]

The plant is a small shrub found along the road sides. It is characterised by soft hair all over, with cordate leaves, yellow flowers and light yellowish brown bark. It is a source of fibre of better quality than jute. Roots, leaves and seeds are slightly bitter in taste and are used in medicine. The juice of the plant mixed with the juice of Borassus flabellifer is used in elephantiasis for local application. The mucilaginous leaves are used as a demulcent and their infusion is given in fever as a refrigerant. These are also used in dysentery and for poulticing ulcers. A decoction of the leves is said to possess emollient and tonic properties.

The roots are considered to possess astringent, diuretic and tonic properties, an infusion of these is given in urinary diseases, bilious disorders, gonorrhoea, cystitis, strangury haematuria, nervous disorders such as hemiplegia, sciatica and in facial paralysis. The root bark powder is used to relieve frequent micturition and leucorrhoea. The ethanolic extract of the roots showed significant anti-inflammatory activity without any significant antipyretic activity. Seeds are used in bowel complaints such as piles, colic and tenesmus due to their demulcent and laxative properties. Ethanolic extract of the plant exhibited antiprotozoal activity against Entamoeba histolytica strain STA and depressed B.P. in cats and dogs.

The plant contains various alkaloids such as - phenethylamine, S-(+)-N_b-Methyltryptophan methyl ester, hypapherine and quinazoline alkaloids: vasicine, vasicinone and vasicinol, in addition to choline and betaine.[154,155] The total alkaloid content of the whole plant was reported to be 0.085%, maximum amount being in the seeds. The seeds also contain fatty oil, steroids, phytosterols, resins, resin acids, mucin and potassium nitrate. The non-oxygenated fraction of the petroleum ether extract of seeds contains sterculic and malvalic acids along with a usual group of fatty acids while the oxygenated fraction contains coronaric acid.[156] The seed oil is rich source of hydrobromic acid reactive fatty acids.

iii. Sida rhombifolia Linn.

Sida rhombifolia Linn. (Fam: Malvaceae), commonly known as "Mahabala"/"Bala", is a small erect under shrub with stellate hairs and variable shapes of leaves. Seeds are black and smooth. Flowers are yellow, axillary or solitary. This species is polymorphic, comprising a number of varieties. These are:

(1) S. retusa Linn. have obovate, retuse or truncate leaves. Pedicels are equally longer or shorter than the petiole.

(2) S. rhomboidea Roxb. have rhomboid, lanceolate serrate leaves. Pedicels are more than half the length of the leaves, jointed at the base.[157]

The plant is regarded to be useful in pulmonary tuberculosis and rheumatism. Ethanolic extract of the plant depresses the activity of the smooth muscles of guinea pig ileum. The plant is used as an antidote for snake bite and scorpion sting and is also tied round the abdomen during child birth. Stems are employed as demulcent, emollient, diuretic, febrifuge and also used internally in skin diseases. Roots are used in the treatment of rheumatism and leucorrhoea. The infusion of the root is given in dysentery and are taken internally during delivery. The ethanolic extract of roots showed significant anti-inflammatory and antipyretic activities in rats.[158] The aqueous extract was found to be non-toxic in mice.[159]

Aerial parts of S. rhombifolia var. rhomboidea contain n-alkanes (C_{15}-C_{35}), longchain alcohols (C_{14}-C_{35}) and a mixture of Δ^5, Δ^7, Δ^8 sterols.[160] Leaves contain amino acids like lysine, histidine, arginine, asparagine, glutamine, alanine, valine, phenylalanine, leucine, aspartic acid, glutamic acid, glycine, serine, threonine and tyrosine; and fatty acids like myristic, palmitic, stearic, oleic and linoleic acids.[160] Total phytosterol content (as cholesterol) was found to be 0.052%. Roots contain 0.054% of alkaloids.

The following are some of the marketed polyherbal formulations of Bala (S. cordifolia) viz.

Abana (The Himalaya Drug Co., Bangalore)

Alpitone (Zandu Pharm. Works. Ltd., Bombay)

Amycordial (Aimil Pharm (I) Pvt. Ltd., New Delhi)

Arthnex (Sagar Pharmaceuticals, Bangalore)

Ashree cordial (Aimil Pharm. (I) Pvt. Ltd., New Delhi)

Ayufal (Shilpa Chem., Indore)

Balant-kadha No.13 (D.K.Sandu Bros,(Chembur) Pvt. Ltd., Bombay)

Blumyn (Vasu Pharm. Pvt. Ltd., Vadodara)

Boniol oil (Seagull Labs (I) Pvt. Ltd., Gurgaon)

Equilin (Aimil Pharm. (I) Pvt. Ltd., New Delhi)

Gestone (Zandu Pharm. Works Ltd., Bombay)

Gynemyn (Myncil Pharm., Varanasi)

Gypan (Pans Labs., Thane)

Herbo Malt (Arya Aushadh Pharm. Works, Indore)

Lucomyn (Jupiter Pharm. Pvt. Ltd., Calcutta)

Meryton (Vasu Pharm. Pvt. Ltd., Vadodara)

Minnitone (Myncil Pharm., Varanasi)

Myncil Ashoka Compound (Myncil Pharm., Varanasi)

Shakti Vikas (Yogi Pharmacy, Haridwar)

Tentex forte (The Himalaya Drug Co., Bangalaore)

Utrosan (Libra Drugs (I) , Pune)

Vinomyn Forte (Myncil Pharm. Varanasi)

Vitalyf (Lifeline Medicare Ltd., Bombay)

The formulations compiled above, containing the selected drugs clearly offer a scope for evaluation and standardization of these for their anti-inflammatory and anti-hepatotoxic activities. The studies are planned to be undertaken first on preliminary screening by subjecting different powders and extracts of these drugs for AIA against Carrageenan induced artificial oedema and hepatoprotective activity against chemical (CCl_4) and drug (paracetamol and rifampicin) induced hepatotoxicities in albino rats being the known biomodels. The extracts which would be found active in these preliminary studies would then be subjected for a detailed investigation on isolation and characterisation of active principles responsible for their activity using reported techniques in order to substantiate inforporation of drugs in phytopharmaca.

1.4 RESEARCH ENVISAGED

The long established systems of traditional medicine have been evolved by systematically recorded human existence over several millenia. Although not strictly based on concepts of modern science, these, nevertheless, are founded on a corpus of organised knowledge manifested in written documents where, even the scientific method of hypothesis, experimentation and confirmation is discernibly revealed. Such organised systems have been subjected to different approaches in the search for either the compounds or their biological activities. The present investigations, therefore, were aimed to evaluate and standardize the therapeutic efficacy of different plant species belonging to genera such as Curculigo, Fumaria, Inula, Moringa and Sida for the claims made under traditional systems for their anti-inflammatory and hepatoprotective activities on the following lines:

1. Pharmacognostic studies
   - (i) Procurement and identification
   - (ii) Macroscopic and microscopic evaluation
   - (iii) Proximate analysis
   - (iv) Estimation of different inorganic metal ions
2. Phytochemical studies
   - (i) Preliminary phytoprofiles of various crude drugs
   - (ii) Preparation of selective extracts and their preliminary chromatographic studies.

3. Biological studies

   (i) Acute toxicity studies

   (ii) Evaluation of anti-inflammatory activity

   (iii)Studies on normal liver function

      (a) Studies on serum parameters

      (b) Studies on urinary parameters

   (iv) Evaluation of hepatoprotective activity

4. Isolation and characterisation of components from effective extracts of different drugs

5. Studies on hepatoprotective activity of the Isolated components

6. Assessment of activity of some marketed polyherbal formulations.