Introduction
1. INTRODUCTION

In India, we have a unique situation of the co-existence of several officially recognised but markedly different systems of medicine. Among them one of the oldest system of medicine of Indian origin is Ayurveda. Though claims are often made of an effective treatment for a specific disease by one system or the other, objective verification is uncommon. The major share of the therapeutic management in Ayurveda comprise of natural products available locally. There is a popular belief among the people of rural India that Ayurveda can provide better treatment for jaundice. Different experimental models are available to determine the effect of any such effective recipe for the treatment of jaundice. Even then, universally the experimental data could not be extrapolated clinically. Conjectural use of liver protecting drugs are available in the market with insufficient evidences, besides, few synthetics or semisynthetics failed to achieve universal acceptance for the treatment of hepatic failure.

Increased sociocultural modernization, detraction and deviation in food habits, use of chemical fertilizers and pesticides are disturbing the biomass. The natural protection to balance the body homeostasis appear to be of great concern to the community. Nature being the greatest economist has provided with different remedial measures, ready at hand only we have to search the golden treasures, surrounding us.

The present scenario of jaundice with hepatitis changed remarkably after the identification of Hepatitis ‘B’ Virus as one of the causative organism. Viral hepatitis, being a disease of variable severity, is a difficult entity, require a sound clinical eye and proper diagnostic management. It represents with high serum bilirubin, transaminases and ultrastructural changes of the organ with grave sequelae$^{1,2}$. Besides, hepatitis ‘B’ virus, more other conogenous viruses A, C, D, and E are also responsible for viral hepatitis. The immunological response by the hepatitis ‘B’ virus infection provided a unique information both in diagnostic and therapeutic management. Search for natural products from traditional sources for the treatment of jaundice was initiated throughout the globe. New drug development for the treatment of hepatocellular damage specially hepatitis ‘B’ virus (HBV) infection gained momentum after 1988 with an Indian herb *Phyllanthus amarus*, showing alteration in the immune profile caused by hepatitis ‘B’ virus$^3$. Though some clinical trials were made with Ayurvedic drugs, like Arogya-wardhani$^4$ and Punarnavadi-kwath$^4,5$, which reveal encouraging results for the treatment of viral hepatitis, but studies on HBV antibody was not done.
From the 18th century onwards, the specific use of *Silybum marianum* fruits for the treatment of liver diseases, disorders of bile duct and spleen was documented. Over the last 20 years, Silymarin, the active principle has been the subject of numerous biochemical, pharmacological and clinical investigations. Silymarin was shown to be effective in chronic liver disease. Controlled, double blind, randomized trials with Silymarin was carried out in patients with acute viral hepatitis, hepatic fibromatosis, micronodular cirrhosis and the results showed definite improvement of liver functions.

Liver plays a vital role in intermediary metabolism and is responsible for detoxification of many foreign compounds by converting these agents to facilitate biliary or urinary excretion. The aetiology of liver disorders have been attributed to various factors viz., nutritional, biochemical bacteriological, viral and environmental conditions.

The most common disease processes affecting the liver are: Infectious hepatitis and serum hepatitis, with damage of liver cells. Acute viral hepatitis usually resolves quickly, and chemical indices of abnormality revert to normal within a few weeks. However, chronic persistent hepatitis occurs in a few patients, in whom plasma aminotransferase activities and plasma bile acids may remain high for months. Acute viral hepatitis, most common in our country, are observed in patients with hepatocellular toxicity induced by drugs (e.g. paracetamol, halothane, carbon tetrachloride, etc.). Cholestasis most often occur due to extrahepatic obstruction. Intrahepatic cholestasis may be prolonged and severe, especially, due to primary biliary cirrhosis or drugs like phenothiazines. Alcoholism, viral hepatitis and prolonged cholestasis are thought to be the most frequent known causes of cirrhosis of liver in India, but in half of the cases no obvious causes are detected. Less often, cirrhosis is associated with metabolic disorders such as Wilson's disease, cystic fibrosis, haemochromatosis or galactosemia. Cirrhosis is accompanied by increased fibrous tissue formation leading to shrinkage of liver, decreased hepatocellular function and obstruction in bile flow. Lastly infiltration of liver which include tumors, most frequently secondary, metastases from cancers of the large bowel, stomach and bronchus, amyloidosis, the reticuloses, tuberculosis, sarcoidosis and abscesses.
1.1 Role of medicinal plants in management of hepatic disorders

Management of hepatic disorders has become a matter of serious concern worldwide and several laboratories are now engaged in identifying effective hepatoprotective molecules\textsuperscript{15-17} from different sources using different experimental models. Despite this tremendous stride in modern medicine for drug development, there are hardly any drug that stimulate liver function, offer protection to the liver damage or help in regeneration of hepatic cells\textsuperscript{18}. A number of herbs are employed in Ayurvedic system of medicine for liver affections. Many of these formulations containing herbal extracts are sold in Indian market for the treatment of various types of liver disorder\textsuperscript{18,19}.

In Ayurvedic system of medicine, a number of Indian medicinal plants for example \textit{Azadirachta indica}\textsuperscript{20}, \textit{Ocimum sanctum}\textsuperscript{21}, \textit{Picrorhiza kurroa}\textsuperscript{22}, \textit{Andrographis paniculata}\textsuperscript{23}, \textit{Cajanus indicus}, \textit{Tephrosia purpurea}\textsuperscript{24}, \textit{Curcuma longa}\textsuperscript{24,25}, \textit{Berberis asiatica}, \textit{Albizzia chinensis}, \textit{Spinacia eleracea}, \textit{Eclipta alba}\textsuperscript{22}, \textit{Phyllanthus niruri}\textsuperscript{22}, \textit{Tinospora cordifolia}, \textit{Ricinus communis}\textsuperscript{26}, \textit{Swertia chirata}\textsuperscript{27} and many others have been mentioned as having hepatoprotective properties and their efficacy in improving liver functions\textsuperscript{28}.

\textit{Phyllanthus emblica}\textsuperscript{29} is a constituent of various multiherbal formulations for liver ailments marketed in India. Liv-52\textsuperscript{30,31} and Jigrine\textsuperscript{32} are similar herbal formulations containing aqueous extracts of different medicinal plants, some of which are known to possess antihepatotoxic properties. Picroliv, extracted from the roots and rhizome of \textit{Picrorhiza kurroa}\textsuperscript{33} has been shown to impart a marked protective activity against various hepatotoxicants, viz, CCl\textsubscript{4}, paracetamol, thioacetamide and β-galactosamine\textsuperscript{34-36}. Ursolic acid is reported to possess a wide variety of pharmacological properties. The hepatoprotective activity of urosolic acid, isolated from leaves of Eucalyptus hybrid \textit{E. tereticornis} has been reported earlier\textsuperscript{37}. Propolis, a resinous wax like substance produced by honey bees has been claimed to be useful in a variety of liver ailments, infectious diseases, arthritis and tumor\textsuperscript{38}. It has been suggested that the therapeutic activities of propolis depend mainly on the presence of flavonoids which are known for their strong scavenging effect on free radicals\textsuperscript{38}.

Some ayurvedic drugs, viz, Kumari-asav, Kumari-kalp, Arogya-wardhini\textsuperscript{34} and Tamra-bhasma\textsuperscript{39} have also shown the same property. Drug like Liv. 52, one of the oldest ayurvedic
formulations, containing a mixture of several herbal extracts has been reported to protect liver 
from the hepatotoxicity induced by paracetamol, anticancer drugs, antibiotics, oral 
contraceptives, alcohol, allyl alcohol and carbon tetrachloride. Silybum marianum is a 
médicinal plant widely used in traditional European medicine. In France, Italy, Germany, 
Hungary and Greece, its roots, leaves and fruits are thought to be efficacious in treatment of 
chronic constipation and in various hepatic diseases such as jaundice, bile stones, hepatitis and 
steatosis. Following the isolation of the active ingredient by Wagner et al., flavone like 
substance, Silymarin was found to be effective against liver intoxication induced by 
CCl₄, thioacetamide, α-amanitin, ethanol, paracetamol and phalloidin in rat and mouse. 
Silymarin, the active compound has been the subject of numerous biochemical and 
pharmacological studies. Antioxidant activity, stimulation of protein synthesis, antidote 
effect, liver lipid metabolism, anti inflammatory, anti allergic, liver protecting activity in acute 
intoxication and chronic intoxication are the few properties of Silymarin which has been 
reported. 

Hepatic dysfunction due to ingestion or inhalation of hepatotoxins (acetaminophen, 
cadmium chloride, ethanol, carbon tetrachloride, allyl alcohol), severe exposure to industrial and 
environmental pollutants, drugs, viral and other diseases, prolonged use of antibiotics are 
increasing worldwide resulting in acute liver damage.

Considering the above facts, we have initiated our study on Cajanus indicus, which is an 
edible herb. Its fruit is used as pulses through out the Indian subcontinent. In rural India, 
specially gangetic plains, aqueous extract of the leaves of Cajanus indicus have been extensively 
used for the treatment of jaundice and hepatomegally for many years, but the active principle 
remains unknown.

The work carried out on the isolation and characterisation of the active principle of 
Cajanus indicus by the present candidate is included in the present thesis.

1.2 Immunomodulatory properties of some medicinal plants

Specific immunity has evolved as a highly sophisticated defence mechanism of higher 
organisms. Cell-mediated and humoral immune responses have a high level of specificity
directed to antigenic epitopes expressed on molecular components of infectious agents, foreign (transplant) or transformed (cancer) cells, or even autologous cells (auto immunity). In Ayurvedic system of medicine, a large numbers of Dravyas Rasayan (drug based modalities), which constitutes of many herbs. These drugs are considered to promote health, longevity, intelligence, immunosurveillance and body resistance against infections and diseases. Phyllanthus embilica, Withania somnifera, Bacopa monnieri, Allium sativum, Asparagus racemosus, Sida rhombifolia, Picrorhiza kurroa are very popular as rasayan drugs in Ayurvedic literature, possessing immunomodulatory functions. An increasingly important role is being attributed to various herbal product. Many proteins purified from plant seeds, for example concanavalin A, phytohaemagglutinin (PHA), wheat germ agglutinin, pokeweed mitogen and some fungal immunomodulatory proteins (Fip) isolated from Volvariella volvacea, Ganoderma lucidum and Flammulina velutipes have been shown to induce a cascade of events leading to cell activation, proliferation, production of lymphokines. Recent investigations have shown that defined, non-toxic doses of galactoside specific mistletoe lectin (mistletoe lectin-I, a constituent of clinically approved plant, Viscum album L. extracts) have immunomodulatory potencies. The obvious ability of certain lectins (e.g. mistletoe lectin-I) to activate non-specific defence mechanisms support the assumption that lectin carbohydrate interactions may induce clinically beneficial immunomodulation. Viscum album L. (mistletoe) extracts are widely used in adjuvant cancer therapy and are especially suggested to mediate an anti-tumours effect. It was reported that synthetic immunomodulator muramyl dipeptide (MDP) protected hepatic cells from acrolein, chloroform and CCl4 induced hepatotoxicity as tested using isolated rat hepatocytes. Studies on Azadirachta indica have been evaluated on some non-specific and specific aspects of immunity in mice. Some polyherbal formulations which are widely practised in Indian system of medicine, e.g., Septilin has been reported to possess antibacterial, anti-inflammatory and anti-exudative properties. It is extensively used in the treatment of several acute or chronic infections.

Liver diseases like infectious hepatitis, chronic and acute viral hepatitis are often accompanied by secondary infections. Prolonged use of antibiotics suppress the immune system, both T and B cells. Attempts are made to immunomodulate immunosuppressed conditions. In clinical medicine, immunostimulants have a potential role in immunodeficiency
disorders, chronic infectious diseases and cancer, particularly disorders involving the lymphatic system. Chronic infectious disease like viral hepatitis produces a suppression in the T-cell and as a result generalised resistance to infections in an individual is suppressed. As mentioned earlier, the plant Cajanus indicus is used for the treatment of jaundice and various liver diseases. It is already reported that, potent hepatoprotective drugs like, Picroliv from Picrorrhiza kurroa, Phyllanthus species, Silymarin from Silybum marianum possess immunomodulatory function but the mechanism of action remains unknown and unexplained. In view of this, it seemed possible that prolonged use of Cajanus indicus may be effective in preventing the secondary infections of lungs and gastrointestinal tract by enhancing body immunosurveillance.

The present study which is included in this thesis, also evaluates the role of Cajanus indicus as a hepatoprotective agent in different models of in vitro and in vivo studies. To substantiate the possible role of the above plant material, besides, usual biochemical and histological parameters, immunomodulatory role of the above plant was also studied.

1.3 Hepatic metabolism of foreign compounds

The liver has been credited with the responsibility for detoxification. Appreciation of the protective role of the liver, however, has been tempered in recent years by the recognition that some “detoxifying” changes may result in harmful rather than beneficial products. This aspect of hepatic metabolism of foreign compounds is of particular relevance to mechanisms of hepatotoxicity and hepatocarcinogenicity. Viewed broadly, all foreign compounds are potentially toxic. Liver playing the important role in metabolism of many drugs, by converting them to polar, water soluble metabolites which can be excreted in bile and urine. The enzyme systems responsible for the metabolism of foreign compounds are located in the integral parts of smooth endoplasmic reticulum (SER), attached to the lipid layers of its membranes. Metabolism usually involves two types of reaction. The oxidative reactions of Phase I by enzymatic machinery called the mixed function oxidase system (MFO) and it pivotal component, cytochrome P450. In Phase II type of reaction, the Phase I metabolites which may be hepatotoxic are conjugated with polar molecules, for example, glucuronic acid or glutathione. Phase I reactions are likely to yield as transient species, products that can damage the liver or other organs. A Phase II reaction, however, may yield a product with enhanced toxicity, as in the
formation of sulphate ester at the N-OH formed in the Phase I metabolism of 2-acetylaminofluorene.\textsuperscript{75,76} Ethanol undergoes only a Phase I type reaction, mainly catalyzed by the cytoplasmic enzyme, alcohol dehydrogenase, but to a minor degree it is probably metabolised by the microsomal ethanol-oxidising system (MEOS).\textsuperscript{74} Its ultimate products CO\textsubscript{2} and H\textsubscript{2}O, of course do not require a Phase II type reaction for excretion. Its intermediate product, acetaldehyde, is an evanscent toxic molecule.

\begin{figure}[h]
\centering
\begin{tikzpicture}
  \node (RH) {RH};
  \node[below of = RH, yshift = 0.5cm] (inactive) {Inactive Metabolite};
  \node[below of = inactive, yshift = 0.5cm] (excreted) {Excreted};
  \node[above of = RH, yshift = 0.5cm] (active) {Active Metabolite};
  \node[above of = active, yshift = 0.5cm] (conjugate) {Conjugate};
  \node[below of = conjugate, yshift = 0.5cm] (excreted2) {Excreted};
  \draw[->] (RH) -- (active);
  \draw[->] (RH) -- (inactive);
  \draw[->] (inactive) -- (excreted);
  \draw[->] (active) -- (conjugate);
  \draw[->] (conjugate) -- (excreted2);
\end{tikzpicture}
\caption{Schematic relationship of phases I and II of drug (RH) metabolism.}
\end{figure}

[A]

\[\text{Drug} \xrightarrow{\text{MFO}} \begin{cases} \text{(b) Nontoxic Metabolite} & \text{Excretion} \\ \text{(a) Toxic Metabolite} & \text{Liver injury} \end{cases} \]

(b) Acet-GSH

\[\text{Glucuronide} \rightarrow \text{GSH-conjugate} \rightarrow \text{Excretion} \]

[B]

\[\text{AAP} \xrightarrow{\text{MFO}} \begin{cases} \text{(a) Toxic Product} & \text{Necrosis} \\ \text{(b) Acet-GSH} \end{cases} \]

\[\text{Conjugation Glucuronide} \rightarrow \text{Mercapturic Acid} \]

(a) Enhancement of pathway increases toxicity
(b) Enhancement of pathway decreases toxicity.

Fig. 1.2: Illustration of basis for active metabolite leading to necrosis when detoxifying pathways are outstripped.

Hepatic metabolism of drugs has traditionally been regarded as detoxification. It may, however, be a toxification role, converting nontoxic molecules to toxic products. The reactions of the first phase are performed mainly by the MFO system, but some are performed by other enzymes, such as the flavoprotein amine oxidase\(^7\). Drug metabolizing activity can be strikingly enhanced by pretreatment with one of a large number compounds and inhibited, by one of a
number of agents. The inducing agents consists primarily of two groups. One group, exemplified by phenobarbital, induces the activity of cytochrome P450 and cytochrome with reductase and enhances the metabolism of a large number of compounds. The other group, exemplified by 3-methylcholanthrene (3-MC) has a much more limited effect.77,78

Drug metabolism is also importantly affected by species, age, sex, endocrine status, diurnal variation, diet, stress and disease. The most useful variations for study of mechanisms and as potent determinants of toxicity are imposed by chemical induction or inhibition and by species and age effects.

Other pharmacokinetic factors play an important role in disposition of xenobiotics. Their involvement in toxicity is less clear than their importance in the therapeutic use of foreign compounds.

1.3.1 Metabolism and toxicity of carbon tetrachloride

Animals of a species well supplied with the enzyme system responsible for the metabolism of CCl4 are susceptible to the hepatotoxic effects of this agent. The neonatal rat, adult chickens are resistant to CCl4 hepatotoxicity; are also virtually unable to metabolise it.78 Toxicity for the rat is enhanced by enzyme induction with phenobarbital or DDT and decreased when cytochrome P450 system is inhibited.79,80 Enhancement of metabolism increases the amount of active metabolite formed and the hepatotoxicity, while inhibition of metabolism decreases the proportion of CCl4 converted to CCl3* (a free radical), and therefore, the toxicity of a given dose. Injury produced by CCl4 seems to be mediated by a reactive metabolite-trichloromethyl free radical (•CCI3) - formed by the homolytic cleavage of CCl4, or by an even more reactive species - trichloromethyl peroxy free radical (CICOO•) - formed by the reaction •CCI3 with O2. This biotransformation is catalysed by a cytochrome P450 - dependent mono-oxygenase.80,81

The relationship of metabolism to toxicity is somewhat complex. While Phase I reactions convert some xenobiotics to products that are more reactive than the original molecule, Phase II reactions convert these active intermediates to nontoxic conjugates.82 Production of hepatic injury depends on the dose of agent, the proportion converted to a toxic product and the tissue levels of that metabolite which develop.81,83 The tissue levels depend on the balance between
rate of production of the metabolite and the rate at which it is accepted into the detoxifying phase of metabolism or finds other avenues for removal. 

1.3.2 Metabolism and toxicity of acetaminophen

Acetaminophen toxicity provides another example of the relationship between toxifying and detoxifying pathways. Most of the molecule is exerted as conjugates of glucuronate and sulphate.

A minor pathway involves cytochromes P450 oxidation of acetaminophen to N-acetyl-p-benzoquinone imine (NAPQI) and catechol metabolites. Initial detoxification of NAPQI occurs by conjugation with reduced glutathione and subsequently exerted as mercapturic acid. Enhancement of the MFO mediates conversion to the toxic metabolite, to a degree that exceeds available tissue GSH for detoxification, or depletion of GSH enhances toxicity; whereas repletion of GSH or provision of exogenous agents that bind the metabolite inhibits the toxicity. If hepatic reduced glutathione is depleted by the detoxification process, NAPQI can cause a cascade of events that result in hepatocellular death.

1.3.3 Metabolism and toxicity of β-galactosamine HCl (GALN): Mechanism of injury

β-galactosamine is first reported by Keppler et al., 1968, as a cause of hepatocellular injury. GALN has been found to cause acute necrosis. On prolonged administration it leads to cirrhosis and hepatocellular carcinoma. The hepatic injury induced by GALN is an extremely interesting experimental model. According to Decker and Keppler, the lesion resembles that of viral hepatitis. The resemblance of GALN induced hepatic injury to galactosemia of humans suggests that GALN may serve to study the relationship between defective galactose metabolism and the development of hepatic disease.

Induction of hepatic injury in experimental animals results from the metabolism of GALN in liver and the consequent effect on nucleic acid metabolism. Galactosamine enters the pathway for galactose metabolism after which there are two hypothetical alternative pathways. Both lead to the production of UDP-glucosamine. The latter compound, unlike the UDP-glucose, which is produced by galactose metabolism, cannot serve as a uridylate donor in the uridyl transferase reaction. Accordingly, the formation of UDP-hexosamine from GALN is a
metabolic "blind alley" which leads to the trapping of uridylate. The rate of trapping exceeds the capacity of the adult rat liver to generate uridylate. Accordingly, it leads to a striking decrease in the concentration of UDP-glucose, UDP-galactose, UTP, UDP, UMP and UDP-glucuronate. This occurs despite the increased synthesis of uridylate that is provoked by GALN metabolism. The toxicity thus appears to be a form of "high-output failure" of uridylate biosynthesis. The critical level or threshold below which deficient hepatic UTP levels can lead to hepatic injury appears to be 25 or 30% of normal. Thus, the rapidly growing liver of the neonatal rat and the regenerating liver of the adult rats are less susceptible than the liver of the normal adult.

13.4 Hepatotoxic effects of ethanol

The liver disease seen in alcoholics encompasses three main, related entities: steatosis, alcoholic hepatitis and cirrhosis. In addition, hemochromatosis and hepatic carcinoma are seen in association with alcoholism. Steatosis is the initial histologic manifestation of alcoholic liver disease; cirrhosis is the terminal lesion. Alcoholic hepatitis appears to be a stage in the development of cirrhosis. Hemochromatosis is an uncommon and porphyria cutanea tarda a rare concomitant syndrome of the liver disease of alcoholic patients. Primary carcinoma of the liver may be a complication of the cirrhosis or have an etiologic association with alcoholism even in the absence of cirrhosis.

\[
\begin{align*}
\text{ALCOHOL} & \rightarrow \text{STEATOSIS} \rightarrow \text{STEATOCIRRHOSIS} \rightarrow \text{CIRRHOSIS} \\
\text{MALNUTRITION} & \rightarrow \text{STEATONECROSIS} \\
& \rightarrow \text{(ALCOHOLIC HEPATITIS)}
\end{align*}
\]

Fig. 1.3: Histogenesis of cirrhosis due to alcoholism.

Fatty liver, the most common hepatic lesion associated with alcoholism, is found in 70 to 100 percent of all patients taking excessive amounts of alcohol. A lesion of only minor clinical importance per se, its chief import is as the first histologic evidence of the adverse effects of
alcohol on the liver, recognizable by light microscopy. The degree of steatosis can range from fatty change in only a few cells to involvement of almost every hepatocyte. The end of the road of the alcoholic which begins as a fatty liver is cirrhosis. The histology is that of fibrosis and regenerating nodules which lead to architectural distortion.

Fig. 1.4: Pathogenesis of chief clinical features of cirrhosis.
1.3.5 Ethanol and drug metabolism

Ethanol is apparently metabolised mainly by the cytosol enzyme, alcohol dehydrogenase (ADH). A significant fraction, however, estimated at approximately 25 percent, is metabolised by the MFO. The microsomal capacity to metabolise ethanol has been termed the microsomal ethanol oxidising system or MEOS. Both the ADH and MEOS pathway yield acetaldehyde as a toxic intermediate which is ultimately oxidized to CO$_2$ and water. The acetaldehyde is thought to cause a host of deleterious effects, including enhanced lipid peroxidation and damage to mitochondrial and other cellular membranes; depletion of glutathione, depletion of vitamins and trace metals, especially pyridoxine, vitamin A, zinc and selenium; and decreased transport and secretion of proteins through inhibition of the polymerization of tubulin. This view can account for the engorging of hepatocytes with protein, fat and water that progresses to the necrosis and fibrosis found in cirrhotic livers, as well as the various metabolic disturbances observed in alcoholic individuals.

Alcohol being a hepatotoxin is clear from the epidemiologic, experimental and clinical evidence. The toxic role of ethanol in the pathogenesis of liver disease, production of hepatic steatosis, necrosis, alcoholic hepatitis and cirrhosis seems evident.

1.4 An introduction on Cajanus indicus

Pigeon Pea - *Cajanus cajan* Mill sp.; English Cajan pea; pigeon pea; Hindi Arhar; Family Leguminosae (Papilionaceae) Synonym Cajanus indicus.

*Cajanus indicus* synonym *Cajanus cajan* (Linn) Mill Sp. is a Leguminous plant, belonging to the family Leguminosae and sub family Papilionaceae. First domesticated in Asia or Africa and is now widely cultivated in the tropics and subtropics. It is particularly grown in the East Indies; India and West Indies. In India, it is chiefly grown in Madhyapradesh, Bihar, Andhrapradesh, Uttarpardesh, Maharashtra and Mysore. Grown mostly as a ‘Kharif’ crop and used in the form of ‘dal’. This is the second important pulse crop of India. There are two main varieties grown in India (i) *Cajanus cajan* variety bicolor D.C. (Arhar) and (ii) *Cajanus cajan* variety flavus D.C. (Tur.). Arhar comprises of late maturing, large bushy plant, woody under shurb with pinnately trifoliate leaves, bearing purple streaked, yellow flowers and dark coloured pods, each having 4-5 seeds. The fruit is a linear, flat, obliquely acute pod. Most of the cultivated types are annuals with varying heights. A few types which are biennials and
perennials are also found. All types under both varieties have $2n = 22$ chromosomes. The flowers are mostly self-pollinated. Natural crossing to a extent of 65% has also been recorded$^{106,107}$. Both immature and ripe seeds are used as human food and is a good source of protein. Leaves and twigs are used as fodder. The pericarp and husk, separated in threshing are used as cattle feed.

The species is adaptable with regard to climate but thrives best in dry tropics. Its roots penetrate deep into the soil and are considered to be helpful in improving the soil condition especially in parts where the soil are exhausted.

The plant is of important medicinal value and in folklore medicine, its usefulness in various types of liver diseases has been recommended$^{108,49,51}$. Seeds and leaves pastes are used to check secretion of milk, when applied over mammae$^{69}$. Even the seeds are referred to be useful in snakebite$^{49}$.

Some studies on the analysis of pulse without husk and analysis of husk has been made. A study on pigeon peas (Arhar) mosaic virus strain on the extractable leaf protein of Arhar, Cajanus cajan L. (Milli sp.) was also done$^{109}$. Antifungal isoflavones have been isolated from fungus infected stem of Cajanus cajan$^{110}$. Some studies on the proteins of pigeon pea (Cajanus indicus) has been made$^{111}$. The chief proteins of the peas of Cajanus indicus of both bicolour and flavus variety were of two globulin, Cajanin and Concajanin, which account for about 58% and 8% respectively; of the total nitrogen and differed from each other in their sulphur and tryptophan content$^{112}$. Peptization of red gram proteins and their characterization by electrophoresis has been made$^{113}$. Studies on the effect of protein fractions from Cajanus cajan and Dolichos biflorus on the serum, liver and aortic lipid levels in rats fed with a high-fat-cholesterol diet has been made$^{114,115}$. Studies on fixed oils, free organic acids and sugars from some Indian legumes (Cajanus cajan) were made$^{108}$. A highly active preparation of urease was obtained from Cajanus indicus and extraction of the enzyme at optimum pH did not improve the activity$^{116}$. Kinetic behaviour of urease of Cajanus indicus was studied$^{117}$. The enzyme urease (urea amidohydrolase) prepared from the above plant, has been immobilised with glutaraldehyde treated chitin as the solid support$^{116,118}$. 


The immobilised enzyme was characterised by determining the pH profiles and optimum temperature. Effect of glutaraldehyde concentration on the binding of enzyme to chitin was studied\textsuperscript{118}. The storage stability of the chitin-urease system was determined. Several other enzymes were isolated and purified from \textit{Cajanus indicus}. Separation and properties of alpha-galactosidase\textsuperscript{120}, and beta-galactosidase\textsuperscript{120} and beta-L-arabinosidase\textsuperscript{121}, a new enzyme from \textit{Cajanus indicus} were studied.