2.1. REVIEW OF LITERATURE:

Since from the historical era, traditional medicinal plants are playing a vital role in human health care. In India, more than 7000 medicinal plants are used in various tribal regions. However, most of the therapeutic effects of these traditional plants are hardly ever known (Slator TF, 1984). In early days, spices, extracts of spices and medicinal aromatic plants have been considered to treat many diseases. Spices have been used to improve flavors and fragrances of food, while medicinal plants were used for their curative actions. Several spices possess antioxidant properties as well as they are useful in the prevention of food rancidity (Lai P.K. et al. 2004.).

To make the therapeutic use of these traditional plants, it requires strong safety, efficacy and quality data. Then these medicinal plants could be formulated in the form of tinctures, powder, extracts, and also in the form of paste (James ER, 2002).

Hence, it is decided to search such scientifically important plant and to evaluate and establish the therapeutic profile for human use.
LIVER DISEASES:
Liver is a versatile organ of the living animal and human body that is involved in the regulation of inside chemical environment. It is the one of the principal organs that performs extensive range of functions including storage of nutrients, maintance of carbohydrates, homeostatis, secretory and, excretory function. Further, one of the important functions of liver is detoxification of xenobiotics (Friedman L.S. et al., 2002). Numerous hepatotoxins that produce liver injury have been recognized as a most important toxicological problem for decades. Due of exclusive metabolic functions of liver and its relationship with gastrointestinal tract, liver is a noteworthy target organ of toxicity. It is the largest playground for detoxication, and disposition of endogenous substances, and is constantly and extensively exposed to different kind of chemicals like xenobiotics, hepatotoxins, and chemotherapeutic agents. Thus may cause impairment of its functions (Preussmann R. et al. 1978). Therefore, it is treated as a key organ that regulates homeostasis in the body. Further, liver is involved in many of the biochemical pathways related to energy provision, reproduction, growth, fight against various disease conditions, nutrient supply etc (Ward FM et al. 1999).

Not only has the liver performed many of the physiological functions but also to guard against the harmful effects of ingested drugs and chemicals. Though there is scientific advancement in recent years, in the field of hepatology and/or liver diseases still there is increase in liver ailments and diseases. Among the liver diseases, jaundice and hepatitis are accountable for a high rate of mortality (Pang S et al 1992).

The majority of the hepatotoxic chemical compounds damage liver cells mainly by oxidative stress inducing lipid peroxidation and other oxidative damages (Chattopadhyay RR, 2003). Hepatotoxicity is one of very widespread condition resulting into serious incapacities that range from severe metabolic ailments/disorders to even mortality. Hepatotoxicity in most of the cases is due to free radical generation. Free radicals are primary to numerous biochemical processes and represent an essential part of aerobic life cycle and metabolism.
(Tiwari A, 2001). Hepatic damage or liver necrosis or membrane damage leads to higher levels of serum SGOT and SGPT in blood stream. These elevated levels of serum marker enzymes are indicators of serious liver damage (Drotman RB et al, 1978).

Tough there is remarkable advances in contemporary medicine, liver diseases are still major health alarms inspite of the development made in the field of medicine and pharmaceutical sciences. Till date no effective drugs treatment is available that stimulate liver functions. Further, no drug claims to offer protection against liver damage or to regenerate hepatic cells (Chattopadhyay R.R, 2003). Therefore, the unavailability of reliable drugs that claims liver-protective effect in modern medicine, many of the herbal medicinal preparations have been suggested in the management of liver ailments and diseases (Chatterjee T.K, 2000). However, relatively less claimed to show noteworthy effects in liver disorders.
DIABETES:
It is a metabolic disorder that may be chronic in nature and, cause a big challenge worldwide. During diabetes mellitus, there is an elevation in plasma glucose levels. This elevation may result from insulin deficiency or insulin resistance, or both, that leads to abnormality in carbohydrate, lipid and protein metabolism [http://care.diabetesjournals.org/content/27/suppl_1/s72.full]. Diabetes mellitus affects around 4% of the world population and is expected to increase day by day (Peungvichaa D. et al, 1998). At present, the treatment of diabetes involves insulin preparations and a range of oral synthetic anti-diabetic drugs like sulfonylureas, thiazolidinediones, α-glucosidase inhibitors. Such kinds of drugs are prescribed as monotherapy or in combination to achieve superior glycemic control in diabetic patient. Tough the monotherapy or combination is effective the drugs still have several serious adverse effects [New drug targets for type 2 diabetes and metabolic syndrome. Nature 2001, 414:821. ]. Hence, it is demand of hour to contribute in the discovery of newer antidiabetic agents. There is need to focus and study the various natural plant resources that possesses minimal unwanted effects. Reports indicate that natural medicinal plants have a major role in the discovery of newer curative drugs. A report demonstrated that natural plant, Galega officinalis has antidiabetic properties and, then led to the finding and synthesis of oral antidiabetic, metformin (Aiman R, 1970). Thus, scientific study on herbal medicinal preparations that has antidiabetic potential and, having a lesser amount of side effects get substantial attention and provides an opportunity as well as challenge to treat this disease.

Furthermore, medicinal plants play a major role in the new drug discovery process and hence much attention has been given to plants as these are the sources of different biologically active substances such as antioxidants, antibacterials, hypoglycaemic and hypolipidemic agents (Marles RJ et al, 1995).

Till date there is no report indicated that diabetes can be totally curable and, there is no single patient reported total recovered from diabetes. Hence, quickly
rising occurrence of diabetes mellitus is attracting a severe risk to human health throughout the world. In addition, in recent past number of new bioactive compounds isolated from medicinal plants had been reported for their antidiabetic potential in clinical research with more efficacy than traditional oral hypoglycaemic agents. World Health Organization (WHO) had prepared the list of around 21,000 medicinal plants that are used for various medicinal treatment purposes all over the world. Among the listed medicinal plants, over 2500 species are found in India. Further, India produces large number of plants those possess medicinal properties, gifted with a wide variety of agro-climatic environment and hence India is termed as one of the botanical garden of the world (Seth, S. D. et al 2004).

The science of traditional herbal medicine suggests that the numbers of plants are responsible for their hypoglycaemic activities. The literature resources indicates that there are more than sufficient plant species responsible for antidiabetic activity (Rajagopal, K. et al, 2008).

It is well known that plant metabolism is mainly based on presence of carbohydrates. A lot literature indicated sugar lowering potential of various medicinal plants. Many reports indicate therapeutic potential of medicinal plants however; acceptable and proven clinical data is required for the demonstration of full therapeutic effect (Herbert O.C. Mbagwu et al, 2011).

When we look Vedic literatures like Charak Samhita, this reports the use of number of medicinal plants, herbs and the chemical constituents isolated from them. In addition, derivatives prepared from medicinal herbs for the treatment of diabetes mellitus have been reported. Moreover, many of in vivo studies have been conducted to test the hypoglycaemic property of various medicinal plants, and such activities were demonstrated particularly on experimental animals (WL. Li et al, 2004).

Prevalence of diabetes mellitus seems to be more in India, and is rising day by day and hence needs appropriate attention. As per knowledge of traditional medicinal treatment, herbal remedies have been considered suitable for the
management of diabetes mellitus with postprandial hyperglycemia. This is because of the traditional acceptability, accessibility, low expenses and few side effects.

In present days, the treatment of diabetes mellitus involve hyperglycaemia reduction by modern medicines like D-phenylalanine derivatives, meglitinides and α-glucosidase inhibitors, biguanides, thiazolidinediones, sulphonylureas, and more importantly insulin therapy. However, these synthetic and semisynthetic modern drugs have unwanted side effect profile in addition to desired effect. Therefore, the efficacy profiles of these compounds are controversial and hence there is a need of newer compounds for the treatment of diabetes (U.K. Prospective Diabetes Study Group. 1995 and Moller DE. 2001).

It is well known that botanical plants have been the source of medicines for the treatment of various disorders and diseases since many years. Among the reported more important medicinal plants that could be used in various systems of medicine are the Species of the genus Piper (Kirtikar KR et al, 1933, and Parmar NS et al, 1997).

Indian spices that give flavor, color, and aroma to food also have numerous therapeutic properties. Different plants have been reported to reveal antioxidant activity, viz., Ocimum sanctum, Piper cubeba, Allium sativum, Terminalia bellerica, Camellia sinensis, Zingiber officinale Roscoe including some Indian and Chinese medicinal plants. This antioxidant activity is might be due to the presence of flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Aqil F et al, 2006.). Peppers are the important antioxidant sources in the diet of human being as these are rich in vitamin A & C. Such kind of spices and medicinal plants are documented as sources of natural antioxidants and thus play an important role in the management of diseases and disorders (Gayatri Nahak et al, 2011).

Among the Species of the genus Piper, Piper nigrum and Piper cubeba are the two flowering vine in the family Piperaceae. Piper nigrum also called as black
pepper is a monocious or decorous climbing vine native to southern India and Srilanka. It is widely cultivated in these regions and also in other tropical regions. The genus Piper of family Piperaceae with more than 1000 species, is distributed in both hemispheres Piper cubeba Linn., (cubeb), tailed pepper because of the stalks attached, jawa pepper in jawa, and kemukus in Indonesia is a climbing perennial plant (Koul, J.L. et al, 1996).

The fruits of Piper cubeba (Piperaceae) commonly known as Kabab-chini, Java Pepper, Tailed Cubebs and Tailed Pepper. The fruits of Piper cubeba plant having a range of medicinal properties, chiefly used in treatment of Hepatitis. P. cubeba used during confinement as a tonic. P. cubeba also used as an aphrodisiac, used for treatment gonorrhoea. In the mucous membrane of genitourinary organs P. cubeba produced antiseptic, stimulant and diuretic actions. P. cubeba also used in the treatment of vomiting, fever, malaria, sunstroke, rheumatism, asthma, leucorrhoea and peripheral neuritis.

Piper cubeba fruits are usually known as cubeba in Arabic and tailed piper in English. The fruits are one of the ingredients of spices and have a range of medicinal properties (Chopra R. N. et al, 1956.). In conventional medicine the fruits are used as stimulants, appetizers, stomachics, and expectorants. The fruit of piper cubeba is also used to relive the gastric pain, enteritis, and diarrhea. In addition, they are used in the treatment of inflammatory diseases. Furthermore, piper cubeba fruits demonstrated pain and inflammation reducing capacity in laboratory animals [Choi E.M. et al, 2003), which is attributed to the antioxidant activity of some isolated chemical constituents [Nahak G. et al, 2011).

Moreover, the fruits of piper cubeba have also been used in the treatment of abdominal pain, asthma, diarrhea, dysentery, gonorrhea, enteritis and syphilis (Eisai PT, 1995 and Sastroamidjojo S, 1997) and promising effect in the treatment of hepatitis C virus protease (Januario AH et al, 2002).

In Taiwan P. cubeba plant used for the treatment of diabetes and gonorrhoea.
In Indonesia, P. Cubeba is local medicines used for the treatment dysentery, other intestinal disorders and venereal diseases.

In China, P. Cubeba fruits used as stomachic and carminative and also used for the treatment of vomiting, indigestion, abdominal disorders and amoebic dysentery. P. cubeba is also used for the treatment of coughs, sinusitis, sore throats, bronchitis, and genitourinary infections.

The fruits of this plant were reported for free radicals scavenging ability, antioxidant activity, antidiabetic, hepatoprotective, anti-inflammatory and antinociceptive activities.
Aboul-Enein HY et al, (2011):
Had studied and examined *Piper cubeba* isolates for anti-oxidant action viz. magnitude of *Piper cubeba* capabilities to search free radicals, DPPH, hydroxyl radical (HO) and superoxide anion radical (·) in different type of systems.

Adriana PF et al, (2007):
Identified mutagenic latent of *Piper cubeba* seeds simple extract by comet assay and micronucleus test on male, mice and rats. The micronucleated polychromatic erythrocytes and DNA damage are increased statistically, therefore genotoxicity in mice and rats of *P. cubeba* seed extracts confirmed.

Had studied and examined the pyridine class alkaloid i.e. piperine is available in rich amount in *Piper nigrum* and *Piper cubeba* having family of Piperaceae. It is commonly used in preparation of various herbal cough syrups and used as anti malarial, anti inflammatory and anti leukemia. Ethanol extract of *Piper cubeba* having high antioxidant activity.

Studied the three medicinal plants of anti-inflammatory i.e. *Piper cubeba* (fruit), anti-allergic i.e. *Physalis angulata* (flower) and analgesic potential i.e. *Rosa hybrid* (flower) which was identified by paw edema induced with help of carrageenan, ear edema tempted by arachidonic acid and arthritis persuaded by formaldehyde method in mice whereas anti-allergic activities identified by DNFB prompted contact hypersensitivity reaction and analgesic activities studied by hot plate test in mice.

Silva MLA et al, (2007):
Investigated crude ethanol extract of *Piper cubeba* seeds (-)-cubebin against oral pathogens. Increases the antimicrobial activity of Co- group at C9 and substitution with polar groups in aromatic rings i.e. dibenzylbutyrolactone compounds.
Mohamed STS et al, (2008):
Studied hepatoprotective effect of *Annona squamosa* in isoniazid with rifampicin persuaded hepatotoxic model. In this study significantly decrease in total bilirubin, ALP, AST, ALT and γ-GT and increase in total protein level. The effect of toxicity in liver is decreased by the extract but not completely cured when compared with standard drug silymarin.

Studied liver protective action contrary to isoniazid and rifampicin tempted hepatotoxicity in rats of methanolic extract of floras of *Bombax ceiba* L. (MEBC).

Investigated hepatoprotective action in CCl₄ induced hepatic impairment in Wister albino rats of extract of aerial parts of *Jatropha gossypifolia*.

Gayatri G et al, (2011):
Studied hepatoprotective prospective against silymarin as a standard crude drug in carbon tetrachloride induced Wistar rats model for 7 days of ethanolic extracts of *Stachytarpheta indica* (whole plant).

Investigated the liver protective action of different extracts of *Psidium guajava* on liver by erythromycin-induced liver toxicity in albino rats. Lower dose of aqueous extract produced hepatoprotective action whereas hepatotoxic action at higher dose.

Zakaria ZA et al, (2011):
Investigated the hepatoprotective potential of MARDI-produced, dried, fermented and processed virgin coconut oils against paracetamol-induced liver damage of rats.
Investigated liver protective potential of aqueous and alcoholic extract of *Anisochilus Carnosus (L)* leaves against Rifampicin induced hepatotoxicity.

Studied hepatoprotective potential of aqueous and methanolic extracts of *Capparis decidua* stems by CCl4-induced hepatotoxicity in rats. Aqueous as well as methanolic extracts of *C. decidua* stems significantly decrease in levels of AAT, SAAT and bilirubin.

Karthikeyan M et al, (2011):
Investigated hepatoprotective potential of ethanolic extract of *Spermacoce hispida* Linn by CCl4 induced liver toxicity in rats. Hepatic functions like SGOT, SGPT, ALP and bilirubin was determined.

Studied hepatoprotective action of Petroleum ether (60-80°) extract of *Ficus carica* in rats. For assessment of hepatoprotective action, determination of Serum levels of GOT, bilirubin and GVT, along with liver weights was done. Petroleum ether extract of *Ficus carica* shows hepatoprotective action of extract with significant biochemical, functional and histological changes.

Lahon K et al, (2011):
Investigated hepatoprotective activity of alcoholic leaf extract of Sacred /Holy Basil, means Green Tulsi (*Ocimum sanctum*), (family - Lamiaceae ), using silymarin as a standard drug.

Studied the liver protective Nutmeg actions in lipopolysaccharide (LPS) plus D-galactosamine (D-GalN) induced hepatotoxicity. Due to presence of essential oil compound Myristicin in Nutmeg produced most powerful hepatoprotective activity.
**Manokaran S et al, (2008):**
Investigated Areva lanata hydroalcoholic extract liver protective action in paracetamol induced liver damage in rats. The hydroalcoholic extract produced significant hepatoprotective activity against reference standard drug Silymarin.

**Shyamkumar B et al, (2010):**
Studied hepatoprotective action of diethyl ether extract of *Coccinia indica* leaves for liver toxicity in rats. Diethyl ether extract of *Coccinia indica* leaves shows hepatoprotective action at dose 400 mg/kg against standard i.e. silymarin at 125 mg/ kg dose.

**Gnanasekaran D et al, (2012):**
Studied hepatoprotective potential of *Indigofera tinctoria* extract on hepatic cells of human using SRB assay and MTT assay.

**Jamshidzadeh A et al, (2006):**
Studied hepatoprotective potential of several hydroalcoholic extract containing different concentrations of dried powder of leaves of *Cichorium intybus* in CCl₄ induced liver toxicity. The extract show hepatoprotective effect only at doses of 50 and 100 mg/ kg whereas higher concentrations are less effective.

**Mohamed BA et al, (2008):**
Studied ameliorative potential of aqueous extract of flesh (*Phoenix dactylifera* L.) and ascorbic acid against hepatotoxicity in rats. In which0 ameliorative potential of extract of flesh of dates for Thioacetamide - induced liver damage.

**Aghel N et al, (2011):**
Studied hepatoprotective latent of extract of *Ficus carica* leaf in CCl₄ persuaded hepatotoxicity in rats. Extract of *Ficus carica* leaf shows less destruction of liver cells, no fibrosis and moderate inflammation with better protection hepatic damage at dose of 200 mg/kg.
Patil K et al, (2011):
Investigated hepatoprotective potential of C. trigonus fruit extracts on CCl₄ induced hepatotoxicity.

Studied liver protective potential of extract of P. lactaiflora and A. membranaceuos on liver damage tempted by a Bacillus Calmete-Guerina and lipopolysaccharide in mice. The extract of P. lactaiflora and A. membranaceuos innocently reduced in elevation of serum transaminase activities, degree of liver damage and level of nitric oxide with protective effect by antioxidant properties.

Patere SN et al, (2009):
Investigated anti-oxidant and hepatoprotective action of polyherbal formulation Normeta on hepatic toxicity induced in rats by 10–30% alcohol, thermally oxidized oil of 15% of diet and carbonyl iron for 30 days. Antioxidant and metal chelating activity of Normeta showed by anti-oxidant studies i.e. DPPH, Nitric oxide and Ferric chloride methods. Normeta shows good hepatoprotective potential when physico-metabolic parameters comparaed with silymarin as an standard drug.

Studied Olanzapine prompted hepatopathy models used for showing putative liver protective agents. Olanzapine prompted hepatopathy models are one of the best model for screening against silymarin as standard drug. In this model silymarin has failed to provide any hepatoprotection.

Mankani KL et al, (2005):
Investigated hepatoprotective potential of methanolic extract of Pterocaorpus marsuopium stem barks against CCl₄ prompted liver toxicity in male Wistar rats.
**Pornpen P et al, (2007):**
Studied liver protective action of aqueous extract of *Phyllanthus amaorus* Schuam in ethanol-induced hepatic rat. Aqueous extract of *Phyllanthus amaorus* Schuam showed increased % MTT lessening assay with decreased release of transaminases in rat hepatic cells cured using ethanol. Histopathological studies showed favourable hepatoprotective role by antioxidant activity.

**Pattanayak S et al, (2011):**
Investigated liver protective action of flavonoids extract of *Cajanus scarabaeoides* (L) in paracetamol befuddled albino rats.

**Sangameswaran B et al, (2008):**
Evaluated liver protective action of *Andrographis lineata* having family Acanthaceae for CCl₄ prompted hepatic injury in rats.
Evaluated Gymnema sylvestre of family Asclepiadaceae shows anti-diabetic action in alloxan induced diabetic and normal rat. Gymnema sylvestre suggestively decreased fasting blood glucose level, serum triglyceride and cholesterol and increase in level of serum HDL in diabetic rats.

Jude EO et al, (2011):
Evaluated hypolipidaemic and antidiabetic potential of Croton zambesicus ethanolic root in alloxan prompted diabetic rat. Extract of Croton zambesicus significantly reduced echelons of fasting blood glucose level, triglycerides, serum cholesterol, LDL, VLDL and increase in levels of HDL cholesterol, also produced nephroprotective action in diabetic rats.

Evaluated antidiabetic action of Madhuhari churna in alloxan induced diabetic rats and also investigated effects of oral glucose tolerance and effect on glycogen level. Orally administered extract of Madhuhari churna significantly produced action on falling glucose and improved glucose patience.

Investigated different extracts of Acacia catechu Willd of family Leguminosae shows anti-hyperglycemic potential in a alloxan prompted diabetic rat. Acacia catechu created considerable anti-hyperglycaemic action and also produce dose dependent hypoglycaemia.

Had studied and evaluated hypoglycemic activity of different type of extracts of 30 medicinal plants in alloxan induced diabetic rats. From the medicinal plants such as Azadirachta indica, Brassica juncea, Pterocarpus marsupium and Allium sativum, showed hypoglycemic activity by stimulation of insulin through direct effect on pancreas.
Hatapakki BC et al, (2005):
Investigated ethanolic and aqueous extracts of flowers of *Cassia auriculata* Linn shows antidiabetic activity in rats having alloxan induced diabetes. Ethanolic and aqueous extracts significantly reduced serum glucose level in diabetic rats. By using column chromatography β sitosterol was isolated from ethanol extract.

Had studied and evaluated antioxidant and antidiabetic action of ethanolic extract of whole plant of *Mollugo nudicaulis*. The extract significantly decreases in a levels of blood glucose, triglycerides, cholesterol, lipid peroxidation, liver glycogen, LDL, serum creatinine level and liver marker enzymes i.e. AST and ALP.

Mohammed FA et al, (2010):
Evaluated antidiabetic activity in alloxan produced diabetic rats against methanolic extracts of *Vinca rosea*. In diabetic rats 500 mg/kg dose of extracts of *Vinca rosea* produced significant activity against diabetes than dose of 300 mg / kg, and development in a body weight, lipid profile and regeneration of β-cells of pancreas.

Investigated antidiabetic potential in streptozotocin prompted diabetic rat against methanolic extract of *Dorstenia picta*. The extract consiting 75 mg/kg dose considerably condensed blood glucose along with raised serum cholesterol and triglycerides.

Investigated antidiabetic action of leaf of *Poongamia pinatta* in alloxan prompted diabetis in albino rats. *P. pinatta* ethanolic extract and aqueous extract significantly produced antidiabetic activity by using glibenclamide as an standard drug.
**Vivek Kumar S et al, (2010):**
Evaluated antidiabetic properties of ethanol extracts of leaves of Ficus Glomerata Linn in alloxan monohydrate induced diabetes in albino rats. Ethanal extract of Ficus glomerata significantly produced dose dependent anti-hyperglycemic activity on 4<sup>th</sup>, 7<sup>th</sup> and 10<sup>th</sup> day post treatment. The blood glucose level is reduced 100 mg / kg dose but the results were statistically insignificant. The Glibenclamide is used as a standard drug.

**Balasubramanian A et al, (2006):**
Had studied and evaluated hypoglycemic effect in alloxan prompted diabetic rat of Casearia esculenta by using tolbutamide used as standard drug. At oral dose of a Casearia esculenta significantly reduced blood glucose level.

**Nagaraja P et al, (2010):**
Investigated stem extracts of Tinospora cordifolia (Willd.) shows anti-diabetic activity in streptozotocin induced diabetic albino rats. The stem extracts of Tinospora cordifolia produced significant anti-diabetic activity at different dosages i.e. 200 and 400 mg/kg. The Lante Zinc Insulin used as a standard drug. Tinospora cordifolia produced its anti-hyperglycemic action through several peripheral mechanisms like by increasing glycogen hepatic storing or minimizing glucose release or decreased glycogen phosphorylase activity and increased hepatic glycogen synthase.

**Laxmi V et al, (2010):**
Evaluated hypoglycemic potential different extracts of Cassia occidentalis in alloxan prompted diabetic rat. The extracts of Cassia occidentalis formed significantly changes in serum cholesterol, serum protein, triglyceride and body weight.
Syed MA et al., (2005):
Studied aqueous extracts of leaves of *Terminallia catapa* Linn. having family Combretace shows significant anti-diabetic potential in alloxan-induced diabetic rats.
2.2. **AIM & OBJECTIVE:**

Pharmacological properties of several medicinal plants are being investigated all over the world due to recent scientific developments with low toxicity and affordable cost. The liver is the second biggest organ of the human body and a major site for drug metabolism in the human body with severe risk of injury along with number of complications by drugs, toxic chemicals, viruses or infectious materials. As per recent review of WHO, due to hepatic diseases lots of people die every year. The most universal diseases of liver are hepatic encephalopathy, portal hypertention, hepatic failure, cirrhosis, cholestasis, hepatitis and tumours like hepatoma. Now a day’s individuals are aware regarding role of free radicals in common diseases and benefits of antioxidants to body. To treat many disorders such as hepatic and diabetes alternative medicines provide a holistic approach like Ayurveda, Siddha and Unani with success. The present study designed for investigation of phytochemical investigation, screening of antioxidant, hepatoprotective and antidiabetic activity of fruits of *Piper cubeba*. Primary objectives of this work as per given below:

1. Identification, collection and authentication of fruits of *Piper cubeba*
2. Preliminary extraction of the fruits of *Piper cubeba* using petroleum ether and ethanol and fractionation with ethanolic extract
3. Preliminary phytochemical investigation of ethanolic extract of fruits of *Piper cubeba*
4. Evaluation of Antioxidant activity,
5. Evaluation of Hepatoprotective activity in CCl₄ induced hepatotoxicity model and Ethanol induced hepatotoxicity model and
PLAN OF WORK:

1. Plant materials Collection, identification and authentication. *Piper cubeba* fruits was obtained from Mahavir Ayurvedic Bhandar, Mumbai, India and authenticated from authorized person.

2. Extraction
   Dried powder of fruits of *Piper cubeba* extracted with petroleum ether (60-80°C) and ethanol by soxhlet method.

3. Phytochemical Investigation
   Characterization of fruits of *Piper cubeba* extracts for various phytoconstituents by chemical test for determination of different chemical constituents.

4. Antioxidant activity by using spectrophotometer assay
   - DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) free radical scavenging activity,
   - Reducing power assay
   - Hydroxyl Radical Scavenging Activity
   - Nitric Oxide Radical Scavenging Activity
   - Hydrogen peroxide radical scavenging activity

5. Pharmacological evaluation of fruits of *Piper cubeba*
   a. Procurement of Experimental Animals.
   b. Toxicity study as per OECD guidelines
   c. Screening for Hepatoprotective activity by Carbon tetrachloride induced toxicity models.
   d. Screening for Hepatoprotective activity by ethanol induced toxicity models.
   e. Screening for Antidiabetic activity by using Alloxan Hydrate induced diabetic’s model
6. Various biochemical parameters are estimated such as,
   a. Hepatoprotective activity:
      • Alanine aminotransferase (ALT)
      • Aspartate aminotransaminase (AST)
      • Alkaline phosphatase (ALP)
      • Total bilirubin (TBil) and Direct bilirubin (DBil)
      • Total Protein (TP)
      • Serum triglycerides (STG)
      • Lipid peroxidation
      • Reduced glutathione
      • Catalase

   b. Antidiabetic activity
      • Estimation of Blood Glucose (By Glucometer)
      • Estimation of Total Cholesterol and HDL Cholesterol
      • Estimation of HDL Cholesterol
      • Estimation of Triglycerides

7. Morphological and histopathological parameters like weight and examination of isolated liver was performed.
2.3. PLANT PROFILE (Piper Cubeba):

A phytotherapeutic approach is the traditional system of medicine can provide many valuable drugs to modern drug development. Searching of uncontaminated API viz. phytochemicals may be costly and longer process. In the treatment of liver diseases number of herbal plants and polyherbal products have been on hand but proven not satisfactory in most of the severe cases. Even though the most of studies was inadequate and incomplete in the experimental assessments of various plants and formulations. Hepatic damage caused by chemical induced at subclinical levels in rodents were tested for determination of therapeutic values. The daily used dietary antioxidants were providing protection to liver from harm caused by oxidative mechanisms of noxious chemical. However, experiments are confidently showing the plants were effective against hepatotoxins such as Andrographis paniculata, Eclipta alba, Picrorrhiza kurroa, Phyllanthus maderaspatensis, Tricopus zeylanicus and Silibum marianum. The plants were produced action against Hepatitis B virus are A. paniculata, P. curroa, E. alba, G. glabra, and P. amarus.

In most of the cases of severe liver damage, liver cells turn in to fibrotic state or die, in which treatment should consist of agents be able to stimulate liver cell proliferation. For development of effective herbal combinations for hepatic disorders cure, plants were evaluated thoroughly for properties such as Antioxidant activity, hepatoprotective activity, antiviral activity (Hepatitis B, Hepatitis C etc), prompt of hepatic regeneration and choleretic action. The possibilities of all desired activities are not available in Single plant. For production of desired activities combination of different herbal extracts / fractions were used for the treatment of severe diseases of liver. Formulation of such herbal products with criteria of efficiency and safety can revitalize hepatic functions. Various parts of plants reported as hepatoprotective along with its active constituents given in Table No. 2.1
Various parts of plants reported as hepatoprotective along with its active constituents:

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of plant</th>
<th>Parts used</th>
<th>Active constituent</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Allophyllus edulis Var eudulis and gracillis</td>
<td>Leaves</td>
<td>C-glycissy Flavones</td>
<td>Hoffman-bohm K et al, 1992</td>
</tr>
<tr>
<td>2</td>
<td>Andrographis paniculata (Acanthaceae)</td>
<td>Whole plant or Leaves</td>
<td>Diterpenoid, andrographole and neoandrographide</td>
<td>Handa SS et al, 1990, Kapoor NK et al, 1995</td>
</tr>
<tr>
<td>3</td>
<td>Aegiceras corniculatum</td>
<td>Stem</td>
<td>n-hexane, ethyl acetate and methanol extract</td>
<td>Roome T et al, 2008</td>
</tr>
<tr>
<td>5</td>
<td>Calotropis procera (Asclepiadaceae)</td>
<td>Flower</td>
<td>Procesterol, Cyclosadol</td>
<td>Buger GT et al, 1989</td>
</tr>
<tr>
<td>6</td>
<td>Careya arborea (Barringtoniaceae)</td>
<td>Bark</td>
<td>Glycosides, Saponins, flavonoids, Tanins and Phenols</td>
<td>Senthilkumar N et al, 2008</td>
</tr>
<tr>
<td>7</td>
<td>Commiphora berryi</td>
<td>Bark</td>
<td>Phytosteroids, Protiens, Gums, Phenolic compounds, Tannins</td>
<td>Shankar NLG et al, 2008</td>
</tr>
<tr>
<td>8</td>
<td>Corydalis saxicola</td>
<td>Whole plant</td>
<td>Dehydrocavidine</td>
<td>Wang T et al, 2008</td>
</tr>
<tr>
<td>10</td>
<td>Curcuma longa (Zingiberaceae)</td>
<td>Rhizomes</td>
<td>Curcuminoides, Curcumin</td>
<td>Somchit MN et al, 2002</td>
</tr>
<tr>
<td>Sr. No</td>
<td>Name of plant</td>
<td>Parts used</td>
<td>Active constituent</td>
<td>References</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------</td>
<td>----------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>13</td>
<td>Fumaria indica (Fumariaceae)</td>
<td>Whole plant</td>
<td>Carbohydrates, Alkaloids, Saponins, Steroids</td>
<td>Waynforth HB et al, 2005</td>
</tr>
<tr>
<td>14</td>
<td>Ganoderma lucidum</td>
<td>Whole plant</td>
<td>Peptides</td>
<td>Yanling S et al, 2008</td>
</tr>
<tr>
<td>15</td>
<td>Luffa acutangula (Cucurbitaceae)</td>
<td>Fruit</td>
<td>Flavonoids, Phenolic glycosides, Carbohydrates, Saponins</td>
<td>Paget GE et al, 1983</td>
</tr>
<tr>
<td>16</td>
<td>Mamording subangulata (Cucurbitaceae)</td>
<td>Leaf</td>
<td>Momordicine, Saponin, Carotene</td>
<td>Ahmad A et al, 2002</td>
</tr>
<tr>
<td>17</td>
<td>Phyllanthus amarus (Euphorbiaceae)</td>
<td>Stems and Leaves</td>
<td>Phenolics and Flavonoids</td>
<td>Faremi TY et al, 2008</td>
</tr>
<tr>
<td>18</td>
<td>Phyllanthus niruri (Euphorbiaceae)</td>
<td>Leaves</td>
<td>Phenolics and Flavonoids</td>
<td>Sabir SM et al, 2008</td>
</tr>
<tr>
<td>21</td>
<td>Silybum marianum (Compositeae)</td>
<td>Above ground parts</td>
<td>Flavonolignans, Silybin</td>
<td>Wang M et al, 1996</td>
</tr>
<tr>
<td>22</td>
<td>Swertia chirata</td>
<td>Aerial</td>
<td>Ligman, Syringaresinol</td>
<td>Chakravarti AK</td>
</tr>
</tbody>
</table>
### Table 2.1: Parts of plants along with active constituents reported as hepatoprotective

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of plant</th>
<th>Parts used</th>
<th>Active constituent</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Gentianaceae)</td>
<td>parts</td>
<td></td>
<td>Maiti BR et al, 1994, 1997</td>
</tr>
<tr>
<td>23</td>
<td>Trichilia roka</td>
<td>Roots</td>
<td>Polyphenol, Limonoids</td>
<td>Germano MP et al, 2001</td>
</tr>
<tr>
<td></td>
<td>(Meliaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Tylophora indica</td>
<td>Leaf</td>
<td>Alkaloid, Steroid, Saponin, Triterpenes</td>
<td>Gujarati V et al, 2007</td>
</tr>
<tr>
<td></td>
<td>(Asclepiadaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.1. ORIGIN:
Piper Cubeba live in Sumatra, Southern Borneo and other isles in Indian Ocean, rising without farming in plantations, also cultured in coffee farmsteads of Java. Before completely developed, the fruit are collected and used as a medicine when fruits were completely dried. The fruit or berries are rough, globular in shape, greyish in colour, a bit of lighter-colour than black pepper, pleasant and aromatic odour, and warm, vicious, and rather camphoraceous taste. The cortical ration is stripper and a smaller amount tender than black pepper, and *piper cubeba* comprises a solid sphere-shaped seed having whitish and oily characteristics. Cubebs are having “spherical” with size 4 or 5 mm, reticulately crumpled, outer colour is blackish-gray and inside whitish and echoing; odour is strong and spicy; taste is aromatic and pungent (cubeb-piper-cubeba/ – [online]).

2.3.2. TAXONOMY: (wikipedia/Cubeb)

- **Kingdom**: Plantae.
- **Division**: Magnoliophyta.
- **Class**: Magnoliopsida.
- **Order**: Piperales.
- **Family**: Piperaceae.
- **Genus**: Piper.
- **Species**: P. cubeba.

2.3.3. VERNACULAR NAMES:

- **Sanskrit**: Chinorana, Renuka, Kakkola, cinatiksna
- **Hindi**: Kabab-chini, Sheetal-chini, Kabachini
- **Marathi**: Pimpli, Mothi
- **Tamil**: Takkolam, Valmilaku, Kanakamilaku
- **Malyalam**: Val-milaku
- **Telugu**: Toka-miriyalu, Halava-miriyalu
- **Urdu**: Kabab-chini, Shital-chini
- **Kanada**: Gandha menasu, Balmenasu
2.3.4. CHEMICAL CONSTITUENTS: (wikipedia/Cubeb)

The fruits consist of essential oil wherein mainly monoterpenes, sesquiterpenes and lignans. The monoterpenes such as β-elemene, carene, 1,4-cineol and 1,8-cineol, sabinene and α-thujene; sesquiterpenes such as cubebol and germacene; b-caryophyllene, copaene, d-cadinene, α- and β-cubebene, and a small number of lignans including the dibenzylbutyrolactone lignan i.e. (-) cubebin. (Wiart. et al, 2006).

The active chemicals are persuasive and discerning inhibitors of cytochrome CYP3A4 which are (-)-dihydrocubebin, (-)-clusin, (-)-dihydroclusin, (-)-yatein, (-)-hinokinin and 5 - methylenedioxyphenyl lignans (Usia et al, 2005).

Other chemical constituents are allo-aromadendrene, asarone, bicyclosesquiphellandrene, calamene, cesarone, cubebic acid, cubebinolide, cubenol, epicubenol, gum, g-humulene, ledol, g-terpinene, limonene, linalol, myrcene, nerolidol, ocimene, resinoids, sabinol, safrole-α-murolene, α-pinene, α-phellandrene, α-terpinene, α-terpineol, β-bisabolene and β-pinene (Dr. Duke’s).

Volatile oil can be acquired by distilling cubebs with water. Cubebene in liquid quota is main constituent having chemical formula is C_{15}H_{24}. Cubebene is a blue-yellow or pale green thick liquid with a hot woody, odour is slightly camphoraceous. Cubebene on keeping or after rectification with water deposits rhombic crystals of camphor of cubebs.

In 1839, cubebs consist of active crystalline substance Cubebin (C_{10}H_{10}O_{3}) discovered by Eugène Soubeiran and Capitaine. Cubebin primed from cubebene or pulp remained subsequently condensation of oil. The piper cubeba contains fatty oils, gum and malates of calcium and magnesium, also contains cubebic acid about 1% and resin about 6%.

2.3.5. PHARMACOGNOSTIC STUDY OF Piper Cubeba FRUITS:

MACROSCOPY OF LEAF:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Grayish-brown to black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>pleasant and aromatic</td>
</tr>
<tr>
<td>Taste</td>
<td>Strong, spicy, slightly bitter and constant</td>
</tr>
</tbody>
</table>
Shape: Smaller prolate - elliptically shaped and surface is smoother

The stalked berries are a little bit larger having wrinkled surface, dried pericarp is wrinkled; seed is hard, oily and white in colour.
Photograph 2.2
2.3.6. TRADITIONAL USES:

The *Piper cubeba* (Piperaceae) fruits are used in treatment a variety of diseases. The fruits possess therapeutic effects and can be used to treat hepatic and diabetic disorders.

*Piper cubeba* used as a tonic during confinement. *Piper cubeba* used as an aphrodisiac. In mucous membrane of the genitourinary organs *Piper cubeba* produced stimulant, antiseptic and diuretic actions, also used in treatment of gonorrhoea. *Piper cubeba* used in the treatment of vomiting, fever, malaria, rheumatism, asthma, sunstroke, leucorrhoea and peripheral neuritis.

In Taiwan *Piper cubeba* used in treatment of gonorrhoea and diabetes. Its continuous use may produce diarrhoea.

In Indonesia, *Piper cubeba* is local medicines used for the treatment dysentery, venereal disease and other intestinal disorders.

In China, *Piper cubeba* fruits used as stomachic and carminative, also used in treatment of vomiting, indigestion, abdominal disorders and amoebic
dysentery. The *Piper cubeba* fruit is also used for the treatment of coughs, bronchitis, sinusitis, sore throats and genitourinary infections. It is a vata and kapha suppressant. *Piper cubeba* is stimulant for human body tissues because of bitter taste and astringent properties. *Piper cubeba* also improves circulation due to astringent taste. *Piper cubeba* fruits clean up RT and expel further mucus from tract. *Piper cubeba* is a good aphrodisiac agent and give relief from various menstrual disorders.

According to ayurveda *Piper cubeba* comprises of Gunna (properties) – tikshan (sharp), ruksh (dry) and laghu light Rasa (taste) - katu (pungent) and tickta (bitter), Virya (potency) – ushan (hot).

### 2.3.7. OTHER PHARMACOLOGICAL ACTIONS:

*Piper cubeba* principally used in treatment of gleet and sub-acute gonorrhea. The fruits generally used in treatment of Hepatitis and diabetes. Used in atonic dyspepsia due to its warming action, but with true tonics mainly as a stomachic, and by covering the disagreeable taste of copaiva use in gonorrhea is important for their action on the kidneys. are rapidly and diffusely stimulant, specifically influencing the kidneys and bladder. At higher doses it increased cerebral circulation leads to headache and dizziness. By macerating (or percolating) procedure Tincture of cubebs are prepare. *Piper cubeba* is diuretic, antiseptic, carminative, stimulant and expectorant. *Piper cubeba* is helpful in treatment of chronic bronchitis, also used in dysentery and digestive ailments. On mucous membranes of respiratory tracts and urinary tracts *Piper cubeba* produces local stimulating action. *Piper cubeba* plant is used in treatment of prostate infections, urethritis, cystitis, and leucorrhea. For the treatment of influenza virus and Bacillus typhosus Cubeb oil is used.

**Trypanocidal activity:**
Chagas disease cause by Trypanosoma cruzi infection and this disease is very difficult to control. *Piper cubeba* contains five (-)-cubebin derivative compounds shows an extraordinarily effective cradle for gaining new lead composites to treat Chagas' disease.
Analgesic, anti-inflammatory and antinociceptive activity:

*Piper cubeba* shows anti-inflammatory analgesic and antinociceptive activity due to its derivatives; (-)-O-acetyl cubebin, (-)-O-methyl cubebin and (-)-O-dimethylethylamine cubebin (30 mg/kg).
2.4. **STANDARD DRUG (Silybum Marianum):**

*Silybum Marianum* is regularly known as a “milk thistle”. For different liver diseases *Silybum marianum* is a favourable drug, due to its superior safety profile, oral effectiveness and available in India at affordable price. The efficacy of *Silybum marianum* is in liver diseases by reinstatement of hepatic functions and regeneration of hepatic cells. Due to its superior calibration, quality control *Silybum marianum* show superior than polyherbal formulations (Jacobs BP et al, 2002). The advanced new approach is in addition to liver, other organs also protected by Silymarin (Pradhan SC et al, 2006).
2.4.1. TOXONOMY:

- Kingdom : Plantae
- Order : Asterales
- Family : Asteraceae
- Genus : Silybum
- Species : S. Marianum

2.4.2. DESCRIPTION:

*Silybum marianum* is a stout thistle; its growing height is 1 - 3 meters in rocky type of soils, the flowering heads is large having colour is purple. The characteristics of leaves are distinct white “milky” veins, due to this characteristic this plant is commonly known as “milk thistle” (Grunewald J et al, 2000).

**Flower and Fruit:**

The inflorescences are purple in colour, solitary and large. The flower heads are composite and fairly nodding. The perigone shape is globular. The petals
are different types; outer petals at the base are tough and inner petals are taper to a slender point, then spread and finish at a horny tip. It consists of only tubular florets. The fruit are brown in colour, spotted and glossy in nature, with a white tuft of hair (Grunewald J et al, 2000).

**Leaves, Stem and Root:**

The plant grows is a straight stem up to 1 - 3 meters i.e. 70 to 150 cm. The upper side leaves are clasping and lanceolate and lower leaves are indented-pinnatisect. The leaves having yellow thorns at margin and white colour spots nearby the ribs of leaf (Grunewald J et al, 2000).

**Habitat:**

The plant is resident from Europe.

**Other names:**

Mediterranean Milk Thistle, Marian Thistle, Mary Thistle

2.4.3. **ACTIVE CONSTITUENTS:**

The mixture of 4 flavonolignan isomers present in the Silymarin, the isomers are silybin (60 to 70 %), silychristin (about 20 %), silydianin (about 10 %) and isosilybin (about 5 %). The silybin is the chief constituent in the Silymarin it’s about 60-70 %. The chemical formula is C_{25}H_{22}O_{10}.

The similarity of silymarin structure is understood and accountable role for protein synthesis activity. Bioavailability of silybin is increase by Silipide is the silybin - phosphatidylcholine complex. Silymarin is present in high amount in fruit and seeds and also found in the whole plant. Silybum seeds contain betaine and essential fatty acids, betaine produced hepatoprotective action and essential fatty acids produced anti-inflammatory effect (Lee JI et al, 2006).

2.4.4. **PHARMACOKINETICS:**

The Silymarin is available in sugar coated tablet form or in the form of encapsulated standardized extract. Silymarin is water insoluble. The Silymarin absorption is low by oral route and generally silybin is recovered from rat bile
in 24 hours. In human beings the Silymarin is excreted in bile about 20 - 40 % of the dose as sulphates and glucoronide conjugate. In experimental animals and in human beings after oral administration of Silymarin peak plasma levels reach within 4 hours – 6 hours and the elimination half life is 6 hours.

2.4.5. EXPERIMENTAL PHARMACOLOGY:
All over the world the Hepatoprotective activity of silymarin is established by number of investigators by using restricted hepatectomy. The commonly used toxic models in experimental animals are carbon tetrachloride induced toxicity, acetaminophen induced toxicity, ethanol induced toxicity, D-galactosamine induced toxicity and *Amanita phalloides* toxin induced toxicity.

2.4.5.1. Hepatectomy:
The Rats which are having restricted hepatectomy in which 70 % of liver will be discarded, the treatment of silymarin showed improved creation of DNA, increased synthesis of RNA, increased synthesis of protein and increased synthesis of cholesterol which regenerate liver. The protein synthesis increased in damaged livers with partial hepatectomy. Silymarin start physiologic controller, subsequently silybin fits in to a specific binding site on the polymerase, which leads to stimulating ribosome formation. Generally Silymarin is able to enter the nucleus and stimulate RNA polymerase I specifically, due to its structural similarity to steroids. Silymarin initiate to suppress nuclear Kappa B (NF-kB) DNA binding activity and its dependent gene expression (Somchit MN et al, 2002).

2.4.5.2. Carbon Tetrachloride:
The assessment of hepatoprotective properties of a drug, carbon tetrachloride (CCl₄) is systematically used from the various chemical agents. Silymarin is complete the normalization of elevated transaminases levels to evaluate activity of polyherbals in CCl₄ induced liver toxicity in rats. In rats with carbon tetrachloride induced cirrhosis, silymarin treatment totally protect against an increase in membrane ratio of cholesterol: phospholipids and sphingomyelin: phosphatidylcholine (Stickel F et al, 2007).
2.4.5.3. **Acetaminophen:**

It is basically NSAID’s but at higher doses of Acetaminophen produced centrilobular necrosis. In paracetamol induced hepatotoxicity silymarin prevented a hepatic cell necrosis in about 87.5 % animals. Therefore, silymarin shows histopathological confirmation of liver protection action by averting liver necrosis or regeneration of cell.

The protection of rats by using Silybin dihemisuccinate (flavonoid of silymarin) in contradiction of hepatic glutathione depletion in case of liver toxicity which was induced by acetaminophen.

2.4.5.4. **Ethanol:**

The severe and long lasting administration of ethanol which leads to hepatic content of GSH is decrease, minimized GSH is a significant biomolecules compared to chemical cytotoxicity. The liver protective action of silymarin has been confirmed in different animals against ethanol- induced liver damage. The various lavels of ALT, AST, γ-GT, and disruption in ratio of condensed glutathione and oxidized glutathione is increased because of ethanol.

Silymarin received group have not been shown any important, silymarin showing protective role against ethanol (Stickel F et al, 2007, Pares A et al, 1998).

2.4.6. **MECHANISM OF ACTION:**

2.4.6.1. **Antioxidant Properties:**

The normal importance of biochemical processes in the body produced by Reactive oxygen species (ROS) system which leads to increased xenobiotics acquaintance. The free radical damage mechanism include ROS - in the cell membrane bilayer persuaded fatty acid peroxidation, which leads to chain reaction of a lipid peroxidation, hence cell sheath injured and producing additional lipids and a proteins oxidation.
2.4.6.2. **Stimulation of Liver Generation:**

Silymarin excites RNA polymerase action by entering into the nucleus by activating accompaniment position on DNA, polymerase usages as pattern for RNA synthesis. In the cytoplasm for the formation of ribosomes, Ribosomal RNA is necessary where protein synthesis takes place. Hence, the amount of a ribosomal RNA is increases which leads to number of ribosomes increases and upsurge in a protein synthesis. The enzymes and structural protein are increases leads to stimulate the restoration of injured cells and synthesis of DNA also increases which leads to increase in mitosis and cell proliferation. This mechanism is most significant pharmacologically consequences in restoration of dented hepatocytes and normal functions of hepatic restoration (James ER, 2002).

2.4.6.3. **Anti-Inflammatory Actions:**

The pharmacological properties of silymarin are inhibitory effect on lipoxygenase and cyclooxygenase pathway leads to leukotriene and prostaglandin synthesis inhibited. The NF-kB is main controller of immune and inflammatory responses. Silymarin is conquer together NF-kB DNA requisite action and reliant on gene appearance tempted by okadaic acid in liver cells surface HEP G2. Silymarin also responsible for mass cell stabilization induction and neutrophil migration inhibition (Trappoliere M et al, 2008).

2.4.6.4. **Antifibrotic action:**

In the Liver fibrosis remodelling of liver structural design which leads to portal hypertension, hepatic insufficiency and hepatic encephalopathy. In this processes complex cell interactions and intermediaries are involve. The liver proliferation parenchymal cells in preliminary stage. In the fibrogenesis the liver stellate cells convert to myofibroblast reflected as essential experience. Silymarin constrains a NF-kB and delays initiation of HSC (Trappoliere M et al, 2008).
2.4.6.5. **ADVERSE EVENTS:**

Silymarin is non-toxic in rats and other animals and it does not produce any toxicity on high and long dosing period, and this can be proved in various investigations. In Human studies silymarin does not shown any side-effects. The commonly used dose of Silymarin in adult is 240 mg / day to 900 mg / day in 2 to 3 distributed doses. Silymarin can produce laxative action due to improved bile flow at higher doses (>1500 mg/ day). Mild sensitive responses may observe, but not severe and sufficient for discontinue the treatment (Luper SND, 1998).