The present investigation tracked down the best evidence, by which to achieve the aim by surviving the past research work. The thorough search process was conducted through electronic data bases in publication related to

- Phytochemical analysis
- Haematological studies
- Enzymatic and non-enzymatic antioxidant activities.
- Hepatoprotective enzymes
- Histopathological studies

2.1. Phytochemical analysis

Plants remain a major source of medicinal compounds. About 20,000 plant species are used for medicinal purposes (Penos, 1983). Seventy four percent of plant derived drugs were discovered as a result of chemical studies to isolate the active substances responsible for their traditional use (Farnsworth and Soejarto, 1991). The plants, especially the higher plants contain a variety of substances, which are useful as food additives, perfumes, and in treatment of various diseases as medicine due to their versatile therapeutic potential (Mukherjee and Wahile, 2006).

*Aegle marmelos*

*A. marmelos* is available from many parts of India and other countries. Available literatures reveal that the whole plant, leaf, fruit,
stem, bark, root and essential oil of fruits of this plant are used in various
diseases. It reduces heat in abdomen used by the tribals from Ranchi and
Hazaribug district of Bihar and Mirzapur district of Uttarpradesh
(Maheswari et al., 1996).

The root powder has been reported as remedy for dyspepsia, gastric
trouble, jaundice and swellings (Rosakutti et al., 2000). The pulp of ripe
fruit is given for chronic stomach disorders (Manorajan Sharma et al.,
2002).

The effect of the A. marmelos extract at a dose of 250 mg / kg was
more effective than glibenclamide in restring the values of diabetic
parameters (Kamalakkannan and Prince, 2004). Dhandapani et al. (2007)
observed ethanoveterinary herbal practices in Thanjavur district, some of
ethanoveterinary important plants include A. marmelos, Casia auriculata,
Mukia scarbrella and Lippia nudifloea.

**Phytochemical constituents**

Many biologically active compounds were isolated from various
parts of A. marmelos. Fresh leaves yield marmelin on distillation which is
yield yellowish-green oil with a peculiar aromatic odour. A sterol
compound Aegelin was isolated from the leaves of A. marmelos. It is a
neutral alkaloid, with one methyl or diethyl groups (Chakravarthi and
A. marmelos has been found to be a phenolic base having an oxazole and a pyridine moiety from UV, IR, NMR and mass spectra from degradative experiments. The leaves were analysed for dry matter, crude proteins, fiber, hemicellulose, cellulose, lignin ash and silica (Chatterjee and Majumder, 1971). Analysis of the leaf oil of A. marmelos afforded the identification of eighteen monoterpenic hydrocarbons (71.85%), four sesquiterpenic hydrocarbons (14.2%) and one oxygenated sesquiterpene (0.78%). Duke (1992) stated the phytochemicals and their biological activities in A. marmelos. It contains P-Cymene, Phellandrene and Skimmianine. P-Cymene is used as Analgesic, Antibacterial, Antiflu, Antirheumatalgic, Antiviral and Herbicide (Garg et al., 1995). The presence of alkaloids in the leaves of A. marmelos, coumarins in the root and stem bark along with others constituents are reported (Das and Das, 1995).

The major constituents of the leaf extract were identified to be tannins, skimmianine, essential oil mainly aryophyllene, cineole, citral, eugenol, sterols, triterpenoids, including lupeol, sitosterol, flavanoids (mainly rutin) and coumarins, including aegeline, marmesin and umbelliferone (Karawya et al., 1980).

The different methanolic extracts of A. marmelos plant parts like leaves, fruit, bark, pulp, flora parts were prepared and screened phytochemically by standard tests. All parts showed the presence of carbohydrates, aminoacids, proteins, anthocyanins, steroids, glucosides and etc. The alkaloidal amides belonging to cinnamide class were isolated
from the leaves of *A. marmelos*. Their chemical transformation and 13CNMR has been reported (Shweta Norender, 2005).

**Eclipta prostrata**

Kirtikar and Basu (1933) reported that the herb *E. prostrata* popularly known as Bhringraj or Bhangra can be used in many indigenous systems of medicine. It is more valuable in cosmetics.


**Phytochemical constituents**

Different types of chemical constituents like alkaloids, glycosides, coumarins, flavonoids, lipids, polyacetylene compounds, triterpenes, steroids, saponins, steroidal alkaloids and etc. have been reported by various workers in different parts of *E. prostrata* from different countries. The environmental factors like geographical sources, season and time of collection may also cause variability in these constituents whereby contradictory reports regarding the presence or absence of certain
constituents are found to exist. The reported compounds are mentioned below.


Roots and aerial parts are reported to contain various types of number of (more than 26 number) Eclalba saponins, triterpene acid glycoside, Eclipta saponin A, Eclipta saponin B and Eclipta saponin D (Zhang and Chem, 1997); oleanolic acid (Zhang et al., 1996); steroids-and steroidal alkaloids-Ecliptalbine, verazine and its derivatives (Abdel-Kader et al., 1998) are reported to be present in E. prostrata.

2.2. Haematological studies

Alcohol has numerous adverse effects on the blood cells and their functions. Over consumption of alcohol can cause generalized suppression of blood cell production and the production of structurally abnormal blood cell precursors that cannot mature into functional cells. Alcoholics frequently have defective red blood cells that are destroyed prematurely, possibly resulting in anemia. Alcohol also interferes with the production and function of white blood cells, especially those that defend the body against invading bacteria. Consequently, alcoholics frequently suffer from
bacterial infections. Finally, alcohol adversely affects the platelets and
other components of the blood-clotting system. Heavy alcohol
consumption increase the risk of stroke (Deitrich and Erwin, 1996).

Latvala et al. (2004) findings suggest that alcohol abuse results in
diverse patterns of hematological effects and affects several cell lines.
Therefore, in patients undergoing bone marrow examinations due to
cytopenias, the probabilities for like findings seem to be different between
alcoholics and non-alcoholics. Information on ethanol consumption
should be systematically included in the clinical assessment of such
patients.

Adeolu et al. (2007) studied the effects of five suspected poisonous
plants of the spurge family (Euphorbiaceae) that is *Alchornea cordifolia*,
*Cnidoscolus acontifolius*, *Phyllanthus amarus*, *Phyllanthus muelleriarus*
and *Securinega virosa*, commonly found in Nigerian pasture were
evaluated in albino rats using crude aqueous extracts for 14 days. All the
extracts were administered orally. Changes in haematological and
biochemical parameters were used as indices of toxicosis. The extracts of
the plants caused a significant reduction in the levels of PCV and
haemoglobin concentration. All except *C. acontifolius* caused a significant
reduction in RBC level. The extract of four plants (*A. cordifolia*
*, C. acontifolius*, *P. amarus* and *P. muellerianus*) caused significant
changes on the total white blood cells when compared to that of the
control. The extracts also caused a significant increase in the levels of
total protein, albumin and AST activity. The extracts of *A. cordifolia*,
*P. muellerianus* and *S. virosa* caused a significant increase in the level of ALT. Only *P. muellerianus* and *S. virosa* produced significant changes in the globulin level.

Nwinuka *et al.* (2008) studied the effects of crude aqueous extract of *Mangifera indica* (Mango) stem bark on body weight and haematological parameters in normal albino rats. The test aqueous extract of the plant was administered to each rat in the group for a period of 14 days. Observations showed that the extract of the medicinal plant have some effects on the haematopoietic system manifested by a positive increase in the levels of PCV (haematocrit), erythrocyte, leukocyte, platelet counts and lymphocytes, while the haemoglobin (Hb) and neutrophil levels were decreased. The test plant also caused an increase in the weights of the rats.

Ajayi *et al.* (2009) investigated the haematological effects of *Allium sativum* (Garlic) on experimental rats exposed to lead for one week. Administration of lead significantly decreased haematological indices, haemoglobin, hematocrit and mean corpuscular volume. Results depicted that administration of lead in rats caused hepatic damage in the animals and that post-lead treatment with *A. sativum* exerted some hepatoprotective effects.

Atta *et al.* (2010) analysed the RBCs counts and Hb concentration, total or differential leucocytes counts in ethanol intoxicated rats and it
showed decreased platelet counts, platelet distribution, width, mean platelet volume and platelet cell ratio.

Sani et al. (2011) investigated changes in haematological parameters of rats fed with prolonged graded doses of *Anisopus mannii*. The limit dose test of up and down procedure as revised by Dixon was employed to determine the acute oral toxicity of the plant. The result revealed that the Median lethal dose of the plant is greater than 3000 mg/kg body weight. The repeated administration of graded doses of the extract showed a significant increase of the packed cell volume (PCV) and red blood cells count on 21st and 28th day post extract treatment. However, there were no significant difference in the total white blood cells and differential leukocyte count in all the treated groups of rats compared to their respective day zero and control group.

The effects of soxhlet extraction of oil from castor seeds (*Ricinus communis*) using n-hexane as solvent on hematological and histopathological properties of albino rats was investigated using standard method. The haematological analysis of the animals blood showed that the extract caused a reduction in the packed cell volume (PCV) from 49.3 to 46.7%. Since the oil has deleterious effects on the organs of the animals used and also reduced the PCV, it is conceivable that when the oil is consumed by humans, it will have the same effect. Therefore, it is advocated that the oil should not be consumed because it causes some liver disorders (Momoh et al., 2012).
2.3. Enzymatic and non-enzymatic antioxidant activities

Antioxidants are the compounds with free radicals scavenging activity and capable of protecting the cells from free radical mediated oxidative stress. The antioxidant compounds can be derived from natural sources such as plants. Antioxidant activity of these plants is due to the presence of flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins. Asteraceae and Rutaceae plants are extensively reported to possess antioxidant activity against a variety of free radicals. Increased exposure to ROS or free radicals is known to cause aging, dementia, diabetes, radiation related musculoskeletal, cardiovascular and several other diseases (Ames, 1994).

Antioxidative defence systems not only counter damage to macromolecules triggered by free radicals generated in aerobic metabolisms of fats, proteins, carbohydrates and xenobiotic metabolism as well as multifactoral oxidative stress but are critical in preventing human diseases including cancer, atherosclerosis, stroke, rheumatoid arthritis and neurodegenerative diseases like Parkinson and Alzheimers and in maintenance of good health.

For a healthy life style, well balanced, wholesome diet and antioxidants supplementation by vegetative mode is being recognized. Present communication discusses the therapeutic role of phytonutrients and dietary antioxidants (vitamin E and C) as well as endogenous antioxidants (SOD, LPO, CAT, GSH, GPx and GST) after proper
isolation, purification, characterization and production of such products as supplements in ayurvedic medicinal formulations.

In ancient times people used spices and herbs in their food as flavouring agents (Beuchat, 1994; Cutler, 1995). These can also be used locally as food preservatives and in folk medicine. Many herbs and spices also possessed free radical scavenging, antioxidant and antimicrobial activities like bactericidal and bacteriostatic.

Today worldwide medicinal plants, spices and herbs have a large number of these spices and herbs in regular use as alternative medicines (Lis-Balchin and Deans, 1996). Lipid peroxidation in fats and fatty foods not only deteriorates their quality and brings about chemical spoilage, but also generates free radicals and reactive oxygen species which are implicated in carcinogenesis, mutagenesis, inflammation, aging and cardiovascular diseases (Shahidi, 1997).

Punicalagin and punicalin, isolated from the leaves of *Terminalia catappa* are used to treat dermatitis and hepatitis. Both the compounds have strong antioxidative activity. The antihepatotoxic activity of punicalagin and punicalin on carbon tetrachloride (CCl₄) induced toxicity in the rat liver was evaluated. Levels of serum glutamate-oxalate-transaminase and glutamate pyruvate transaminase were increased by administration of CCl₄ and reduced by drug treatment. The results show that both punicalagin and punicalin have anti-hepatotoxic activity but larger dose of punicalin induced liver damage. Thus even if tannins have
strong antioxidant activity at very small doses, treatment with a larger
dose will induce cell damage (Lin et al., 1998).

Gyamfi et al. (1999) studied five plants such as Desmodium
adscendens, Indigofera arrecta, Tremaocc cidentalis, Caparis
erthrocarpus, and Thonningia sanguinea for their free radical scavenging
action by their interaction with 1,1-diphenyl-2-icrylhydrazyl (DPPH). Of
these five plants, only Thonningia sanguinea was found to scavenge the
DPPH radical. Lipid peroxidation in liver microsomes induced by H₂O₂
was also inhibited by T. sanguinea. The hepatoprotective effect of
T. sanguinea was studied on acute hepatitis induced rats by a single dose
of galactosamine (GalN, 400 mg/kg, IP) and in mice by carbon
tetrachloride (CCl₄, 25 microl/kg, IP). GalN induced hepatotoxicity in rats
as evidenced by an increase in alanine aminotransferase (ALT) and
 glutathione (GSH) S-transferase activities in serum was significantly
inhibited when T. sanguinea extract (5 ml/kg, IP) was given to rats 12 hr
and 1 hr before GaIN treatment. The activity of liver microsomal GSH S-
transferase, which is known to be activated by oxidative stress, was
increased by the GaIN treatment and this increase was blocked by
T. sanguinea pretreatment. Similarly, T. sanguine pretreatment also
inhibited CCl₄ induced hepatotoxicity in mice. These data indicate that
T. sanguinea is a potent antioxidant and can offer protection against GalN
or CCl₄ induced hepatotoxicity.

Dorman and Deans (2000) analysed the antioxidant and radical
scavenging activities of black pepper (Piper nigrum) seeds. Both water
extract and ethanol extract of black pepper exhibited strong antioxidant antimicrobial, larvicidal and anti-cancer activities.

Natural antioxidants are constituents of many fruits and vegetables, and they have attracted a great deal of public and scientific attention because of their anticarcinogenic potential and other health promoting effects. Recent epidemiological studies have indicated that diets rich in fruits and vegetables and those of selected natural antioxidants such as plant polyphenols, vitamin C and flavonoids are correlated with reduced incidence of cardiovascular and chronic diseases and of certain cancers (Laandrault et al., 2001; Zuo et al., 2002).

Moreover, antioxidant, superoxide dismutase and catalase activities of *Piper cubeba* have been reported (Karthikeyan and Rani, 2003). Antioxidant-based drug formulations from Piper species were used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer’s disease and cancer (Devasagayam et al., 2004).

Manikandan et al. (2005) tested the protective effect of both ethyl acetate and methanolic extract of *Acorus calamus* against noise stress (30d, 100 dBA/4h/d) induced changes in the rat brain. The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and the levels of reduced glutathione (GSH), vitamin C, vitamin E, protein thiols and lipid peroxidation (LPO) are measured for the evaluation of oxidative stress status in discrete regions of the rat brain like cerebral cortex, cerebellum, pons-medulla, midbrain, hippocampus and
The findings indicated that during exposure to noisy environment ROS generation led to increase in corticosterone, LPO and SOD, but decrease in CAT, GPx, GSH, protein thiols, vitamins C and E levels. Both the ethyl acetate and methanolic extract of *Acorus calamus* protected most of the changes in the rat brain induced by noise-stress.

Sivalokanathan *et al.* (2006) evaluated the antioxidant nature of ethanolic extract of *Terminalia arjuna* (EETA) bark on N-nitrosodiethylamine (DEN) induced liver cancer in male wistar albino rats. The enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidants like Vitamin C and Vitamin E levels were determined in all the groups of animals. A significant increase in LPO levels was observed while the levels of enzymatic and non-enzymatic antioxidants were decreased, when subjected to DEN induction. These altered enzyme levels were ameliorated significantly by the administration of EETA at the concentration of 400 mg/kg in drug-treated animals. This protective effect of EETA was associated with inhibition of LPO induced by DEN to maintain the antioxidant enzyme levels.

Dash *et al.* (2007) evaluated the hepatoprotective effect of chloroform and methanol extract (CEIF and MEIF) of whole plant of *Ichnocarpus frutescents* by paracetamol-induced liver damage in rats. The chloroform and methanolic extracts of *I. frutescens* (CEIF and MEIF)
were studied for their hepatoprotective and antioxidant effects on paracetamol (750 mg/kg) induced acute liver damage on wistar albino rats. CEIF and MEIF at a dose level of 250 mg/kg and 500 mg/kg produce significant hepatoprotection by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation, while they significantly increase the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in a dose dependent manner. From this study, it can be concluded that the chloroform and methanol extract of *I. frutescens* is not only an effective hepatoprotective agent, but also possesses a significant antioxidant activity.

The antioxidant activity and hepatoprotective potential of *Cirsium setidens* the widely used medicinal plant and the n-butanol (n-BuOH) fraction of leaves and roots of *C. setidens* had a higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity than the other soluble fractions. The n-BuOH fraction of roots of *C. setidens* had a significant hepatoprotective activity at a dose of 500 mg/kg compared to that of a standard agent. The biochemical results were confirmed by histological observations indicating that *C. setidens* extract decreased ballooning degeneration in response to CCl₄ treatment. The n-BuOH fraction reduced CCl₄-induced liver injury in rats, and transcript levels of genes encoding antioxidant enzymes such as glutathione peroxidase 1 (GPO1), glutathione peroxidase 3 (GPO3) and superoxide dismutase (SOD1) were elevated in the livers of rats treated with this fraction (500 mg/kg). Based on these results, it is suggested that the *C. setidens* extract has hepatoprotective effect related to its antioxidant activity (Lee *et al.*, 2008).
Jain et al. (2009) studied the hepatoprotective activity of ethanolic and aqueous extracts of *Amorphophallus campanulatus* tubers evaluated against carbon tetrachloride (CCl₄) induced hepatic damage in rats. The extracts at a dose of 500 mg/kg were administered orally once daily. The substantially elevated serum enzymatic levels were significantly restored towards normalization by the extracts. Silymarin was used as a standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate that *Amorphophallus campanulatus* tubers have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. The ethanolic extract was found to have more potent hepatoprotective than the aqueous extract. The antioxidant activity was also screened and found positive for both ethanolic and aqueous extracts. This study suggests that possible mechanism of this activity may be due to free radical scavenging potential caused by the presence of flavonoids in the extracts.

Rajalakshmy Menon et al. (2010) evaluated the hepatoprotective and antioxidant activity of ethanolic extract of *Bacopa monnieri* in acute experimental liver injury induced by nitrobenzene in rats. The extract at the dose of 200 mg/kg body weight was administered orally once everyday for 10 days. The increased serum marker enzymes, Aspartate transaminase, Alanine transaminase and alkaline phosphatase were restored towards normalization significantly by the extract. Significant increase in SOD, CAT and GPx was observed in extract treated liver
injured experimental rats. It is concluded that the ethanolic extract of *Bacopa monieri* plant possesses a good hepatoprotective and antioxidant activity.

Antioxidant activity of the fruit of *A. marmelos* was reported. Antioxidant activity and free radical scavenging activity of the ripe and unripe fruit of *A. marmelos* was compared and the result indicated that the enzymatic antioxidants increased in ripe fruit when compared to unripe fruit extract (except glutathione peroxidase). The percentage of free radical inhibition was also high in unripe fruit than that of the ripe fruit (Sharmila and Devi, 2011).

Singhal and Gupta (2012) investigated the antioxidant and hepatoprotective activity of methanolic flower extract of *Nerium oleander* against CCl₄-induced hepatotoxicity in rats. The extract showed potent activities on reducing power, lipid peroxide, DPPH, ABTS, superoxide anion, hydroxyl radical and metal chelation. The histopathological observations supported the biochemical evidences of hepatoprotection.

### 2.4. Hepatoprotective activity

Herbal-based therapeutics for liver disorders used in India for a long time and it has been popularized world over by leading pharmaceuticals. Despite the significant popularity of several herbal medicines in general and for liver diseases in particular, there are still unacceptable treatment modalities for liver diseases.
The limiting factors that contribute to this eventuality are (i) lack of standardization of herbal drugs; (ii) lack of identification of active ingredients, (iii) lack of randomized controlled clinical trials (RCTs) and (iv) lack of toxicological evaluation. The use of natural remedies for the treatment of liver diseases has a long history, starting with Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy.

A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi-ingredients of plant formulations (Handa et al., 1986). In spite of the tremendous advances made, no significant and safe hepatoprotective agents is available in modern therapeutics. Therefore, due importance has been given globally to develop plant-based hepatoprotective drugs, effective against a variety of liver disorders.

Till date there is no effective medicine for hepatic disease which is primarily caused by xenobiotics and hepatitis viruses. Consequently, the control of liver diseases has become a major goal of modern medicine.
The drugs offered by modern medicine for the treatment of liver diseases are corticosteroids and immune suppressants which provide only symptomatic relief mostly without influencing the disease process and their use is associated with the risk of relapse and danger of side effects. In traditional systems of medicine, like Ayurveda, medicinal plants and their formulations are used to cure liver diseases. Some of these plants and herbal preparations have been evaluated for their protective actions against hepatotoxins.

The traditional medical practitioners in Sri Lanka use the mature leaves of the plant *Osheckiao clandra* for its antihepatotoxic properties (Jayaweera, 1982). Pre and post-treatment with the aqueous extract of the leaves of *A. paniculata* revealed protection against alcohol-induced alteration of serum and liver transaminase activities (Choudhury and Poddar, 1983). The ethanol/water extract of *E. alba* counteracted the CCl4-induced inhibition of the hepatic microsomal drug metabolizing enzyme amidopyrine N-demethlylase and membrane bound glucose-6-phosphatase. Pre-treatment of *E. alba* normalized the CCl4-induced decrease of acid phosphatase and alkaline phosphatase activities. Saxena *et al.* (1993) suggest that the hepatoprotective activity of *E. alba* may be regulating the levels of hepatic microsomal drug metabolising enzymes.

Gadgoli and Mishra (1995) evaluated the protective effects of the aerial parts of *Achillea millefolium*, seeds of *Eichorium intybus* and aerial parts of *Capparis spinosa*in CC14 and paracetamol-induced hepatic dysfunction in rats. The aqueous extract of *C. spinosa* was found to be
most effective against CC1₄-toxicity model while the chloroform extract of the plant was found to be most effective against paracetamol induced toxicity model. All the extracts (aqueous, methanol, and chloroform) of these three herbs showed varying degrees of hepatoprotection against the toxicities induced by the two different hepatotoxins.

Methanolic extracts of the seeds of *Apium graveolens* and *Hygrophila auriculata* were proved to be protective against paracetamol and thioacetamide intoxication in rats (Singh and Handa, 1995). Both these herbs reversed the hepatotoxin-induced alterations of various biochemical parameters (activities of transaminases, alkaline phosphatase, sorbitol dehydrogenase, and glutamate dehydrogenase in serum; level of serum bilirubin and hepatic triglycerides). The histopathological pattern of the hepatotoxin-induced liver toxicity of the rats treated with the seed extracts of *A. graveolens* / *H. auriculata* showed a normal pattern of tissues.

The protective effect of aqueous-methanolic extract of *Artemisia absinthium* was evaluated on acetaminophen and CC1₄-induced hepatic injury (Gilani and Janbaz, 1995). The re-treatment of rats with the plant extract (500 mg/kg) prevented the acetaminophen as well as CC1₄-induced rise in serum transaminases. Post-treatment with three successive doses of the extract (500 mg/kg) restricted the hepatic damage induced by acetaminophen, but CC1₄-induced hepatotoxicity was not altered.
Emodin isolated from the stem of *Ventilagoleio carpa* exhibited hepatoprotective effects on CC1₄ and D-galactosamine-induced liver damage. Emodin significantly reduced the activities of SGOT and SGPT. Histopathological examination of the liver also showed the protective efficacy of emodin (Lin *et al.*, 1996).

Lin *et al.* (1997) studied the hepatoprotective effect of various fractions of *Scutellaria rivularis* against CC1₄, D-galactosamine and acetaminophen induced toxicity in rats. CHC₁₃ fraction and Et.OH fractions exhibited the greatest hepatoprotective effects on CC1₄ induced liver injuries, the CHC₁₃ fraction and n-hexane fraction were most effective against Digalactosamine intoxication and the CHCl₃ fraction represented the most liver protective effect on acetaminophen induced hepatotoxicity.

The *in vitro* antioxidant and free radical scavenging properties of the bark extracts of *Anadenanthera macrocarpa, Astroniumunn deuva, Mimosa vernrcosa* and *Sideroxylonobtus folium* were studied using different bioassays by Desmarchelier *et al.* (1999). All the extracts (aqueous and methanolic extracts) were active.

Biochemical and histopathological studies on the effect of the leaves of *Cassia occidentalis* (aqueous-ethanolic extract) on paracetamol and ethanol intoxication in rats revealed its hepatoprotective activity (Jafri *et al.*, 1999). Turmeric antioxidant protein isolated from the aqueous extract of turmeric (*Curcuma longa*) has been found to exhibit
hepatoprotection in CCl₄-treated rats. Decrease in the activities of antioxidant enzymes in liver due to CCl₄ intoxication was nearly normalized on treatment with the protein.

Germano et al. (1999) studied the effect of Milracarpus scaber (decoction of aerial parts) on CC1₄-induced acute liver damage (in vivo and in vitro) in rats. In vivo results showed that pre-treatment with M. scaber reduced the elevated activities of serum GOT and GPT due to CC1₄ treatment. In vitro results indicated that addition of M. scaber extracts to the culture medium reduced the CC1₄ evoked elevation in the activities of SGOT and SGPT. In vitro study also revealed the free radical scavenging properties of M. scaber.

Picroliv, the active constituent isolated from Picrorrhiza kurroa, exhibited protection against ethanol-induced hepatic injury in rats. Ex vivo and in vivo studies showed that picroliv treatment (3-12 mg I kg p.o x 45 days) restored the altered parameters in a dose-dependent manner (Saraswat et al., 1999). The ethanolic extract of P. kurroa was shown to protect against D-galactosamine-induced hepatitis in rats (Anandan and Devaki, 1999). The seed extract of Schisandra chinensis showed protective effect on Phase I oxidative metabolism against CCl₄ induced hepatic dysfunction in rats. The pretreatment with the herbal extract 30 min. before liver intoxication exhibited prominent protection (Zhu et al., 1999).
Bhanwara et al. (2000) studied the effect of aqueous leaf extract of *Azadirachta indica* in paracetamol-induced hepatotoxicity in rats. The liver damage due to paracetamol administration resulted in elevation in the activities of serum transaminases and gamma glutamyl transpeptidase (GGT). The extract of *A. indica* (500 mg/kg) significantly reduced the elevated activities of these enzymes in serum. *A. indica* was also found to be effective in reducing paracetamol-induced liver necrosis as evidenced by histopathological studies.

Bhakta et al. (2001) reported that hepatoprotective activity of the n-heptane extract of *Cassia fistula* (Fabaceae) leaves by inducing hepatotoxicity with paracetamol in rats. The extract at a dose of 400 mg/kg body wt. exhibited significant protective effect by lowering the serum levels of transaminases (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP). The effects produced were comparable to that of a standard hepatoprotective agent.

Ahmed et al. (2002) observed the hepatoprotective activity of the *Apium graeolens* against CCl₄ induced hepatotoxicity in albino rats. The degree of protection was measured by using biochemical parameters like serum transaminases (SGOT and SGPT), alkaline phosphatase, total protein and albumin.

Suja et al. (2004) reported the effect of the methanol extract of *Helminthostachys zeylanica* on carbon tetrachloride (CCl₄) induced liver damage in wistar rats. The results showed that significant hepatoprotective
effect was obtained against CCl_4 induced liver damage, by oral administration of *H. zeylanica* methanol extract as evident from decreased levels of serum enzymes and an almost normal architecture of the liver, in the treated groups, compared to the controls. Thus, the study provides a scientific rationale for the traditional use of this plant in the management of liver diseases.

Dahiru *et al.* (2005) observed the protective effect of the ethanol extract of the leaves of *Ziziphus mauritiana* on CCl_4 induced liver damage. Pretreatment of rats with 200 and 300 mg/kg body weight of *Z. mauritiana* leaf extract protected rats against CCl_4 liver injury by significantly lowering aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase, total bilirubin and lipid peroxide levels compared to control.

The hepatoprotective activity of the leaf extract of *Alchornea cordifolia* a Nigerian plant on acetaminophen induced toxicity *in vivo* has been reported (Olaleye *et al.*, 2006). The antioxidative properties revealed total phenolic content of 0.22 mg/ml and reducing power of 0.062 mg/ml as compared to vitamin E with a reducing power of 0.042 mg/ml. The results concluded that the hepatoprotective activity of this plant on acetaminophen induced liver damage is connected to its antioxidative properties.

The methanolic extract of the leaves of *Ficus carica* was evaluated for hepatoprotective activity in CCl_4-induced liver damaged rats. The
extract at an oral dose of 500 mg/kg exhibited a significant protective effect reflected by lowering the serum levels of AST, ALT, total serum bilirubin, and malondialdehyde equivalent, an index of lipid peroxidation of the liver (Krishna et al., 2007).

Afaf et al. (2008) analysed the role hepato-protective of methanolic extract of *Lepidium sativum* at a dose of 200 and 400 mg/kg was investigated in CCl4 induced liver damage in rats. A significant reduction in all biochemical parameters was found in groups treated with *Lepidium sativum*. The severe fatty changes in the livers of rats caused by CCl4 were insignificant in *Lepidium sativum* treated groups.

Methanol, hexane and chloroform extracts of *Prostechea michuacana* (PM) were studied against CCl4-induced hepatic injury in albino rats. Pre-treatment with methanolic extract reduced biochemical markers of hepatic injury levels demonstrated dose-dependent reduction in the *in vivo* peroxidation induced by CCl4. Likewise, pretreatment with extracts of *Prostechea michuacana* on paracetamol-induced hepatotoxicity and the possible mechanisms involved in this protection were also investigated in rats. The degree of protection was measured by monitoring the blood biochemical profiles. The methanolic extract of orchid produced significant hepatoprotective effect as reflected by reduction in the increased activity of serum enzymes and bilirubin. These results suggested that methanolic extract of PM could protect paracetamol-induced lipid peroxidation thereby eliminating the deleterious effects of toxic metabolites of paracetamol. This hepato-
protective activity was comparable with silymarin. Hexane and chloroform extracts did not show any apparent effect. The findings indicated that the methanolic extract of PM can be a potential source of natural hepatoprotective agent (Rosa and Rosario, 2009).

Mohammad Qureshi et al. (2010) tested the hepatoprotective activity of the ethanolic extract of *Leucas ciliata* leaves extract was evaluated by carbon tetrachloride (CCl₄) induced liver damage model in rats. The extract demonstrated a significant dose dependent antioxidant activity comparable with ascorbic acid. In hepatoprotective activity study, CCl₄ significantly increased the levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and total bilirubin. Pretreatment of the rats with ethanolic extract of *L. ciliata* inhibited the increase in the serum levels of SGPT, SGOT, ALP and total bilirubin and the inhibition was comparable with silymarin (100 mg/kg po). The findings revealed that *L. ciliata* leaves have significant hepatoprotective activity.

Petroleum ether extract of root of *Plumbago zeylanica* was investigated for hepatoprotective activity against paracetamol induced liver damage. In serum total bilirubin, total protein, aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, $\gamma$-Glutamyltransferase, total cholesterol and serum triglycerides were determined to assess the effect of the extract on the paracetamol induced hepatic damage. The study was also supported by histopathology of liver sections. Results of this study revealed that the
markers in the animals treated with paracetamol indicating severe hepatic damage by paracetamol, whereas the blood samples from the animals treated with petroleum ether extract of roots showed significant reduction in the serum markers indicating the effect of plant extract in restoring the normal functional ability of the hepatocytes. The dosage of extract of plant roots used was 300 mg/kg bodyweight of rat. The study reveals that the petroleum ether root extract of *Plumbago zeylanica* could afford a significant protection against paracetamol-induced hepatocellular injury (Kanchana, 2011).

The acetone (*AEAC*) and aqueous extracts (*AQEAC*) of *Adina cordifolia*, belonging to the family of Rubiaceae, were studied for hepatoprotective activity against wister rats with liver damage induced by ethanol. It was found that *AEAC* and *AQEAC*, at a dose of 500 mg/kg body weight exhibited hepatoprotective effect by lowering the hepatic enzymes and also significantly increased the levels of total protein. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of ethanol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by ethanol and blood samples from the animals treated with *AEAC* and *AQEAC* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells against ethanol induced hepatocellular injury. The effects of *AEAC* and *AQEAC* were comparable with standard drug silymarin (Sharma *et al.*, 2012).
2.5. Histopathological studies

Carbon tetrachloride and ethanol are toxic chemicals which cause abnormal changes at cellular, biochemical and molecular level. Kapur et al. (1984) studied the effect of oral pre-treatment with Jigrine on hepatic damage induced by alcohol-CCl₄ and paracetamol in rats. Alcohol-CCl₄ and paracetamol treatment produced increase in serum transaminases, bilirubin, plasma prothrombin time and lipid peroxides in liver.

Flaoyen et al. (1995) stated that seven calves were fed with a mixture of bog plants containing 15 g (wet matter) Narthecium ossifragum per kg liver weight for two consecutive days. Their serum creatinine, urea and magnesium concentrations increased, whereas the serum calcium concentration decreased. Histopathological examination of the kidneys of the 5 calves that were killed revealed tubular epithelial cell degeneration and necrosis. Histopathological examination of the kidneys of the calf dosed with the flower stems revealed severe tubular necrosis and degeneration. It therefore appears that both the toxic principles are present in the flower stems of N. ossifragum rather than in its leaves.

Administration of CCl₄ to rat caused hydrophilic changes mostly in centrilobular area including necrosis, ballooning and blood vessel injuries. Other injuries included were sinusoids and congestion of central vein. Inflammatory cells were also seen in the sinusoid area. Fatty infiltration and fibrosis were also induced with CCl₄ treatment (Shenoy et al., 2001).
Carbon tetrachloride is used as a hepatotoxic chemical in experimental animals. Tissue damage by carbon tetrachloride depends upon the amount of dosage and duration of exposure of the experimental animals to this toxicin, causes dysfunctions of lungs, kidneys, brain, and testis. It can also induce the production of free radicals in blood as well (Szymonik-Lesiuk et al., 2003).

Okada et al. (2003) reported the effect of CCl₄ on liver of rats and observed that CCl₄ causes severe parenchymal damages, delay in regeneration of hepatocytes and prominent proliferation in mesenchymal cells and fibrosis in growth hormone deficient rats as compared to normal rats. Vascular degeneration in centrilobular to mid zonal hepatocytes and necrosis of hepatocytes was also observed. CCl₄ exposure in rats also causes variation at biochemical level. It affects the level of liver marker enzymes in serum, antioxidant enzymes and non enzymatic antioxidant compounds like Vitamin C, E and other compounds which were recently investigated (Kamalakkannan and Prince, 2005).

Azza-Abd and Maguid (2006) detected the effect of colchicine administration on the histology of liver and intestine in albino rats. Colchicine was given with a dose of 3 mg/kg body weight daily for a period of 5 days. Histological examinations were carried out at one, four and seven days of post treatment. Histological examination of liver on one, four and seven days post treatment with colchicine showed sporadic necrosis, loss of hepatic architecture, pyknosis and vacuolations of some hepatocytes, corrugated hepatic portal vein surrounded by large fibrotic
area, edema of portal tract with new bile ductules formation, dilatation and congestion of hepatic sinusoids, multi haemorrhagic areas, hypertrophied hepatocytes with deeply stained shrunken nuclei and mono nuclear cells infiltration replacing focal areas of hepatic necrosis.

The carbon tetrachloride increased serum membrane marker enzymes, such as alkaline phosphatase (ALP), amino transaminase (AST), gamma glutamyl transpeptidase (γ-GT), alanine transaminase (ALT), and biochemical such as bilirubin, total serum protein, globulin and creatinine, while decreased albumin and creatinine clearance showing abnormality of liver and kidney. It has been reported that when liver plasma cells are injured and causing release of cytosolic enzymes in to blood circulation. Quantification of serum enzymes can be a useful criterion to assess the oxidative damage (Jadon et al., 2007).

Jayakumar et al. (2008) reported protective effects of Pleurotus ostreatus. (Oyster mushroom) versus toxicity imposed by CCl₄ in rats. In rats, the CCl₄ significantly exhausted the vitamins E level, GSH and elevated the level of MDA in heart and kidneys. The activities of glutathione peroxidase (GSH-Px), catalase (CAT), and glutathione-S-transferase (GST), and superoxide dismutase (SOD) were reduced in brain, heart, and kidneys of rates exposed to CCl₄.

Khan and Ahmed (2009) reported that administration of CCl₄ (2 ml/kg b.w) in 16 weeks in rats affects the histopathology of testicular tissue which includes complete atrophy of seminiferous tubules,
degeneration of the germ cells and as well as inflammatory cells infiltration. Renal injuries have been found in several investigations. Hepatic injuries were induced in rats with administration of CCl₄. The histopathological changes induced by the treatment of CCl₄ alone caused multi dimensional changes including degeneration of fats, necrosis and sinusoidal dilations were seen. Central vein was also found congested.

Atta et al. (2010) investigated the hepatoprotective effect of ginger, chicory and their mixture against carbon tetrachloride intoxication in rats. It increases the RBCs counts and Hb concentration, total or differential leucocytes counts. However it decreased platelet counts, platelet distribution width, mean platelet volume, platelet larger cell ratio. Methanol extract of ginger and chicory given alone or mixed significantly restored the carbon tetrachloride-induced alterations in the biochemical and cellular constituents of blood.

Sundari et al. (2011) reported ethanolic extract of aerial parts of Sphaeranthus indicus was investigated for hepatoprotective activity against paracetamol induced liver damage. Results of this study revealed markers in the animals treated with paracetamol recorded elevated concentration indicating severe hepatic damage by paracetamol, whereas the blood samples from the animals treated with ethanolic extract of roots showed significant reduction in the serum markers indicating the effect of the plant extract in restoring the normal functional ability of the hepatocytes.
Buncharoen et al. (2012) studied the effects of the ethanolic extracts from the root of *S. aphylla* on blood biochemical, haematological and histopathological indices of albino rats. The results of this study showed no significant differences in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen and creatinine of all treated groups when compared to those of the control groups. Nevertheless, an increase in lymphocytes count was observed in all treated groups. The alterations in liver and kidney tissues of all treated groups showed leukocyte infiltration and haemorrhage in hepatic sinusoids. The significant injury of tissues observed in this study is a sign of the toxicity of *S. aphylla* to mammalian species.

The survey indicated that very limited level of research reported on liver disorders. There is still an immature area of research on phytochemical application to liver disorders due to alcoholism. Much work still remains to be done in this ever growing discipline. More research is required at present and it is a rich field of research with full of promising opportunities.