Herbal drugs play an important role in health care programmes worldwide and there is resurgence of interest in herbal medicines of treatment for various ailments. The World Health Organization estimated that about 80 percent of the world’s population still relies on plant-based medicines for their primary health care (Khalil et al., 2007). Natural drugs have been a part of the evolution of human healthcare for thousands of years. Nowadays, nearly 88 per cent of the global populations turn to plant derived medicines as their first line of defence for maintaining health and combating diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15 per cent of all angiosperms have been investigated chemically and of that 74 per cent of pharmacologically active plant derived components were discovered (Raja et al., 2009). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found in vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswas et al., 2002).

Various medicinal plants have been used for years in daily life to treat various diseases all over the world. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant
medicines as alternatives to synthetic drugs (Nair et. al., 2005). Recently various modern procedures and techniques have been developed for the determination of biological activity of plant extract and bioassay techniques (Ahmad et al., 2002; Zafar et al., 2002). Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003).

Over 50 per cent of all modern clinical drugs are of natural origin and the natural products play an important role in drug development programmes in pharmaceutical industry (Baker et al., 1995). Large numbers of plants belonging to different families have been studied for their therapeutic properties (Bowers, 1976; Cordell, 1981; Stuñness and Cordell, 1987; Mukhtar et al., 2002; Devaki et al., 2012). However, plants such as *Aegle marmelos* and *Eclipta prostrata* belonging to Rutaceae and Asteraceae respectively, with many therapeutic properties, have not been studied for their phytochemical constituents and pharmacological properties and hence the present study has focused on those plants.

### 5.1. PHYTOCHEMICAL ANALYSIS

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Igbinosal et al., 2009). Now-a-days modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (Doughhari et al., 2008), because higher plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity
known as secondary metabolites. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies.

Hence, a thorough validation of herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants (Milne et al., 1993).

In recent years GC-MS studies have been increasingly applied in the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids (Jie et al., 1988). In the present study, qualitative phytochemical analysis of extracts of the leaves of both *A. marmelos* and *E. prostrata* revealed the presence of alkaloids, proteins, phytosterols, tannins and phenols in both the plants. Carbohydrate and flavanoids were absent in *E. prostrata* and saponin was absent in *A. marmelos*. The GC-MS analysis showed totally 33 compounds from *A. marmelos* and 16 compounds from *E. prostrata*. All these compounds
are of pharmacological importance as they possess the properties such as anti-diabetic, antibacterial and hepatoprotective activity.

Flavonoids are reported to exhibit antioxidant activity (Ramanthan et al., 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski, 1988). Alkaloids have been identified to have hepatoprotective and antioxidant activities. The alkaloid has the ability to induce antioxidant enzymes and prevent liver damages (Yoshikawa, 2003). There is, therefore, a reason to believe that the hepatoprotective and antioxidant activities of leaves of test plants could be attributed to the presence of alkaloids as revealed in the preliminary qualitative phytochemical analysis. Terpenoids and alkaloids have been proved to have hepatoprotective activity. The presence of alkaloids and terpenoids in leaf extracts of test plants exhibit hepatoprotective properties could serve as a basis for its traditional use as a medicinal plant. The present study agrees with what was reported by Ghoshal et al. (2006) that alkaloids, terpenoids and lactones are responsible for hepatoprotective activity.

Polyphenolic compounds have an important role in stabilising lipid oxidation and are associated with antioxidant activity (Gulçin et al., 2003; Yen et al., 1993). The phenolic compounds may contribute directly to antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to 10 gm is ingested daily from a diet rich in fruits and vegetables (Tanaka
et al., 1998). The extracts of these two test plants can be used for hepatoprotective activity because of their rich phenolic content.

Saponins are secondary plant metabolites that occur in a wide range of plant species (Hostettmann and Marston, 1995). They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack on liver. The natural role of saponins in plants is thought to be protective against attack by pathogens in liver (Morrissey and Osbourn, 1999). These molecules also have considerable commercial value and are processed as drugs and medicines, foaming agents, sweeteners, taste modifiers and cosmetics.

From the literature survey, it is known that saponins of *A. marmelos* and *E. prostrata* have never been studied for hepatoprotective activity. On the other hand, it is known that all these species are effective in hepatoprotective and antioxidant activities. Finally, this study concludes that the presence of these phytochemicals in *A. marmelos* and *E. prostrata* could be the reason for its hepatoprotective and antioxidant activities. The result of this experiment indicates that these medicinal plants have the potentiality to treat liver diseases and it could be utilized to create a healthy environment.

5.2. HAEMATOLOGICAL STUDIES

Haematology is the study of blood, the blood-forming organs and blood diseases. Haematology includes the study of etiology, diagnosis,
treatment, prognosis and prevention of blood diseases that affect the production of blood and its components such as blood cells, haemoglobin, blood proteins and the mechanism of coagulation.

It is one of the rapidly developing discipline which deals with many aspects of blood affecting diseases such as anaemia, leukemia, lymphoma and clotting or bleeding disorders. Haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelet levels have been determined to detect liver disorders as well as hepatotoxic activities.

Total haemoglobin test was used to determine the amount of haemoglobin in blood. Hgb is the pigment part of the erythrocyte, and the oxygen-carrying part of the blood. Normal values of total haemoglobin in male is 12-17/100 ml and females is 11-15/100 ml. A low haemoglobin level indicates anemia which was observed in ethanol intoxicated albino rats, but aqueous leaf extract of *B. alba* consistently increased the total haemoglobin level in ethanol consumed albino rats (Alada, 2000). Likewise, Bishayee *et al.* (1995) reported that aqueous extracts of fresh tuber roots of *Daucos carota* enhanced the total haemoglobin level on CCl₄-induced acute liver damage. The present study reflected that *A. marmelos* and *E. prostrata* aqueous extracts treated groups significantly increased the total haemoglobin in the ethanol consumed albino rats.
The packed cell volume (PCV) is the volume percentage (%) of red blood cells in blood. It is otherwise known as hematocrit or erythrocyte volume fraction (EVF). It is normally about 45 per cent for men and 40 per cent for women (Williams et al., 2004). It is considered an integral part of a person's complete blood count results, along with haemoglobin concentration, white blood cell count and platelet count. The lower level of the packed cell volume is a danger sign of chronic kidney diseases and an increased risk of liver disorder which observed in ethanol administrated albino rats (Bolarinwa et al., 1991).

In contrast, *Apium graveolens* significantly increased the packed cell volume in ethanol consumed albino rats. Similarly, *Alchornea cordifolia*, a Nigerian plant induced the PCV on acetaminophen induced albino rats (Olaleye et al., 2006). The present study reported that *A. marmelos* and *E. prostrata* aqueous extracts treated groups significantly increased the packed cell volume in the ethanol intoxicated albino rats.

Red blood cells (RBC) or erythrocytes, are the most common type of blood cells which deliver oxygen (O$_2$) to the body tissues via the blood flow in circulatory system (Kabanova et al., 2009). RBC is the count of actual number of RBC's per cubic mm of whole blood. The RBC count is useful in determining problems like anaemia and haemorrhage. Red blood cell indices are Mean corpuscular haemoglobin (MCH); Mean corpuscular haemoglobin concentration (MCHC); Mean corpuscular volume (MCV). The MCV reflects the size of red blood cells.
The MCH and MCHC reflect the average amount of haemoglobin and average concentration of haemoglobin per red blood cell respectively. The lowered level of red blood cell count, Mean corpuscular haemoglobin (MCH); Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) were observed in ethanol administered albino rats (Mitchell, 1966).

On the other hand *Terminalia catappa* seed elevated the level of red blood cell count. Mean corpuscular haemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) were observed in ethanol administrated albino rats (Muhammad and Oloyede, 2009). Same results were observed by Gupta *et al.* (2006) on ethanolic extract of *Chamomile recurita capitula*. In the present study also the aqueous extracts of *A. marmelos* and *E. prostrata* significantly increased the RBC and its indices in ethanol intoxicated albino rats.

WBC count is taken to measure the number of white blood cells. White blood cells are very helpful in fighting against microbial infections. They are also called leukocytes. There are five major types of white blood cells namely Basophils, Eosinophils, Lymphocytes (T cells and B cells), Monocytes and Neutrophils. A low number of WBCs is called leukopenia and it can cause bone marrow deficiency, Collagen-vascular diseases (systemic lupus erythematosus), disease of the liver or spleen were observed in alcohol induced albino rats. In contrast, *Alchornea cordifolia* significantly increased the packed cell volume in ethanol consumed albino
rats. Similarly, *Silybum marianum* induced the WBC count in thioacetamide-induced albino rats (Madani et al., 2008). The present study reported that aqueous extracts of *A. marmelos* and *E. prostrata* significantly increased the WBC count in the ethanol intoxicated albino rats.

Platelets, or thrombocytes are small, irregularly shaped clear cell fragments (i.e. cells that do not have a nucleus), 2–3 µm in diameter which are derived from the fragmentation of precursor mega karyocytes (Maton et al., 1993). The average lifespan of a platelet is normally just 5 to 9 days. They circulate in the blood of mammals and are involved in haemostasis, leading to the formation of blood clots.

The low platelet count can make excessive bleeding, feel tired and fatigued (O'Connell et al., 2008). But aqueous leaf extract of *Ficus carica* consistently increased the total platelets level in ethanol consumed albino rats (Alada, 2000). Likewise, Kanchana et al (2011) reported that aqueous extracts of *Plumbago zeylanica* enhanced the total platelets level on paracetamol-induced hepatocellular injury. The results of the present study reflected that *A. marmelos* and *E. prostrata* aqueous extracts have significantly increased the platelets in the ethanol consumed albino rats.

The present study revealed that the aqueous extract of *A. marmelos* has effectively enhanced the haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin
concentration (MCHC), white blood cells (WBC) and platelet levels than alcohol intoxicated rats. Finally, this study has highlighted that the aqueous leaf extracts of *E. prostrata* and *A. marmelos* could be a potential source in haematological activities.

5.3. ANTIOXIDANT ACTIVITIES

Most living organisms possess enzymatic and non-enzymatic defence system against excess production of reactive oxygen species. However, different external factors such as smoke, alcohol, drugs and aging could decrease the capability of such systems resulting in disturbances of redox equilibrium which leads to the propagation of dangerous diseases (Willett, 1994). Antioxidants are also compounds that inhibit or delay the oxidation of the molecules by inhibiting the initiation or propagation of oxidizing chain reaction. Herbal drugs play an important role in worldwide health care programmes and there is a resurgence of interest in herbal medicines in the treatment of various ailments.

Ethanol consumption has induced alteration in the activities of antioxidant enzyme system. Alcohol reduces the levels of antioxidant enzyme activities and promotes the generation of Reactive Oxygen Species (ROS) that leads to liver damage (Dahiru and Oboidoa, 2008; Shanmugam et al., 2011). Ethanol intoxication disturbs the balance between per oxidants and antioxidants to the extent of inducing oxidative damage to biomolecules leading to cell injury.
Superoxide dismutase (SOD) is the major attractive metalloprotein in the antioxidant family. The increased synthesis of superoxide dismutase against superoxide anion radical production is an adaptive response of the cell to synthesis increased mitochondrial SOD through the stimulation of gene transcription. The enzyme SOD was found to be decreased in ethanol intoxicated rats. This is due to the low level of Zn (a metal constituent of the enzyme SOD) in plasma and liver tissues. The low level of zinc was also found in alcoholic liver cirrhosis (Dahiru and Oboidoa, 2008).

In the present study, significant decrease in the activity of liver SOD in ethanol intoxicated rat was observed. The therapeutic treatment with *A. marmelos* and *E. prostrata* herbal drugs significantly improved the level of SOD in liver. This result indicates that herbal drug promoted hepatoprotection by elevating free radical scavenging activity. Similarly, ethanol extract of *Desmodium adscendens, Indigofera arrecta, Tremaocci dentalis, Capariserythro carpus,* and *Thonningia sanguine* enhanced the SOD level in alcohol-fed animals (Gyamfi *et al.*, 1999). Manikandan *et al.* (2005) also observed that methanolic extract of *Acorus calamus* increased the SOD level against noise stress. Similar results were observed in silymarin treated rats. The SOD content in serum and plasma is more significant in *A. marmelos* than *E. prostrata*.

Lipid peroxidation (LPO) is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotic and aging. Malonaldehyde is one of the end products in lipid peroxidation.
process and estimation of malondialdehyde level is an indication of lipid peroxidation and free radical activity. Lipid peroxides are the products of chemical damage done by oxygen free radicals to the polyunsaturated fatty acids of cell membranes.

The increase in lipid peroxidation, a degradative process of membraneous polyunsaturated fatty acid has been suggested by an increase in malondialdelyde in ethanol induced toxicity in the liver (Karthikeyan and Rani, 2003). In the present investigation, the administration of aqueous extract *A. marmelos* and *E. prostrata* at the therapeutic doses (1 g/Kg. b.wt) showed maximum reduction in lipid peroxides level.

The standard hepatoprotective drug silymarin maintained the decreased lipid peroxidation level to normal limits in the liver. The results indicate that the herbal drugs of *A. marmelos* have a very effective antioxidant activity than *E. prostrata* in ethanol induced liver damage. This is a confirmation with the earlier study where the aqueous extracts of black pepper seeds (*Piper nigrum*) induced the lipid peroxidase enzyme level and decreased the level of lipid peroxides (Dorman and Deans, 2000). Similarly, Amol et al. (2011) reported that ethanolic extract of *Garcinia indica* fruit and decreased the level of LPO.

Catalase (CAT) is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. CAT traps the harmful hydrogen peroxide and converts it into water and oxygen. The activity of catalase was found
to be decreased in ethanol intoxicated rats. The inhibition of catalase activity during ethanol induced toxicity may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cells.

In the present investigation, the administration of herbal drugs *A. marmelos* and *E. prostrata* elevated the catalase activity in the liver tissues and protected than from the free radical induced oxidative stress. Similar results have already been reported by Sivalokanathan *et al.* (2006) where the ethanolic extract of *Terminalia arjuna* bark increased the level of catalase in ethanol intoxicated rats. Dash *et al.* (2007) also evaluated the effect of chloroform and methanol extracts of whole plant of *Ichnocarpus frutescens* increased the level of catalase in paracetamol-induced liver damage in rats.

Glutathione (GSH) is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds, such as free radicals. It is a highly sensitive indicator of cell functionality (Meister, 1991). Glutathione is a major non-protein thiol in living organisms which coordinates the body's antioxidant defence process (Valenzuela *et al.* 1985).

GSH not only protects cell membrane from oxidizing damage, but also helps to maintain the sulphotydryl groups of many proteins.
Irreversible cell damage supervenes when the cell is no longer able to maintain GSH content.

The GSH depletion in hepatic mitochondria is considered as the most important sensitizing mechanism in the pathogenesis of alcoholic liver injury. But, ethanol extract of *Desmodium adscendens*, *Indigofera arrecta*, *Trema occidentalis*, *Caparis erythrocarpus*, and *Thonningia sanguine* enhanced the GSH level in alcohol-fed animals (Gyamfi *et al.*, 1999). In the present study, treatment with herbal drugs, *A. marmelos* and *E. prostrata* had significantly improved the level of glutathione both in plasma and liver tissues. However, the effect was more in *A. marmelos* than in *E. prostrata*.

Glutathione Peroxidase (GPX) enzyme plays an important role in the protection of organisms from oxidative damage. It is an antioxidant enzyme that reduces hydrogen peroxide and lipid peroxide (Knapen *et al.*, 2000). GPx is a selenium dependent enzyme which has high potency in scavenging reactive free radicals. The enzyme converts reduced glutathione to oxidized glutathione while reducing lipid hydroperoxides changed into hydrogen peroxide than water. Low levels of GPx have been correlated with free radical related disorders (Lai *et al.*, 2010). Decreased level in GPx content in serum of alcohol treated rats and is subsequently elevated in aqueous extracts of *Amorphophallus campanulatus*, tubers has been reported (Jain *et al.*, 2009). In the present experiment also, aqueous extracts of *E. prostrata* and *A. marmelos* administered group exposes the
escalated level of GPx content in experimental rats. The GPx content was more in animals treated with *A. marmelos* than with *E. prostrata*.

The Glutathione-S-Transferase (GST) is a group of multifunctional proteins, which plays a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood (Brind *et al.*, 2004). The levels of Glutathione-S-transferase were significantly decreased in ethanol intoxicated rats. In contrast, ethanolic extract of *Bacopa monnieri* intensified the level of Glutathione-S-transferase (Rajalakshmy Menon *et al.*, 2010). This observation perfectly agrees with the earlier reports that antioxidant activities of methanolic flower extract of *Nerium oleander* induced the Glutathione-S-Transferase level in acute liver injury induced by nitrobenzene in rats (Singhal and Gupta, 2012). In the present investigation, the administration of herbal drugs *A. marmelos* and *E. prostrata* elevated the catalase activity in the liver tissues and protected from the free radical induced oxidative stress. This result supports that the antioxidant properties of the drug from *A.marvelos* was excellent when compared with that of *E. prostrata* and standard drug silymarin.

Vitamin E and C are natural antioxidants found in a variety of plant materials. Ascorbic acid is the most powerful antioxidant under physiological conditions. Vitamin E and C has been shown to function as antioxidant at various levels. It is an important water soluble antioxidant and readily scavenges Reactive Oxygen Molecule (ROM), ozone, HNO₃, NO₂, NO and hypochlorous acid (Noroozi *et al.*, 1998). Vitamin C
rejuvenates vitamin E making it as an indirect contributor to the fight against free radical damage in the lipids. These two nutrients can be effective partners in reducing the destructive process of lipid peroxidation (Karagezian and Gerorkian, 1989). It exists mostly in the reduced form which can directly scavenge superoxide, hydroxyl radicals and single oxygen. The ascorbic acid reduces H$_2$O$_2$ to water via ascorbate peroxidase reaction.

Vitamin-E is a chain breaking antioxidant. It can repair oxidizing radicals directly preventing the chain propagation step during lipid oxidation.

In the present research work, the decreased level of these vitamins was observed in ethanol intoxicated rats. This may be due to the high level of oxidative stress during intoxication. The reduced form of glutathione substrate is required for the regeneration of vitamin C, which in turn is necessary for the regeneration of vitamin E. The ascorbic acid functions as an aqueous phase antioxidant.

Therapeutic treatment with the drugs from *A. marmelos* and *E. prostrata* in intoxicated rats significantly increased the level of vitamin E and C through the influence of GSH regeneration. Thus, the herbal drugs exert a beneficial effect in regenerating the GSH through the recycling mechanism of these vitamins. This study reported for the first time that aqueous extract of *A. marmelos* has significant antioxidant activities than *E. prostrata*. Further studies could reveal the exact mechanisms of action responsible for the antioxidant activities.
Finally, this study has highlighted that the aqueous leaf extracts of *E. prostrata* and *A. marmelos* could be a potential new natural source for antioxidant activities.

### 5.4. HEPATOPROTECTIVE ENZYMES

Liver is the most important organ concerned with the biochemical activity in the human body and it has great capacity to detoxicate toxic substances and synthesize useful metabolites (Meyer *et al.*, 2001). Liver plays a central role in co-ordinating various metabolic functions of the body. Chronic consumption of ethanol induces lipid peroxidation causing hepatotoxicity by increasing the free radical formation which in turn increases the level of lipid peroxide in hepatic tissue and causes cell injury (Lieber, 1991). Hepatic damage is associated with the distortion of these metabolic functions (Wolf, 1999).

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Guntupalli *et al.*, 2006). In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders.

There is a growing focus to follow a systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases (Shahani,
Therefore an effective formulation has to be developed using indigenous medicinal plants with proper pharmacological experimental study and clinical trials.

Ethanol is one of the most commonly used hepatotoxins in the experimental study of liver diseases (Johnson and Kroening, 1998). It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration (Shi and Li, 2005). The hepatotoxic effect of ethanol is mainly due to its active metabolite, trichloro methyl radical (Srivastava et al., 1990). This activated radical, bind covalently to the macromolecules and induces lipid peroxidation and forms lipid peroxides which produce damage to the membrane (Mujeep et al., 2009). The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase which are cytoplasmic in location and released into circulation after cellular damages was the clear indication of the loss of functional integrity of the cell membrane (Saraswat et al., 1993). Amino transferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine aminotransferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream (Chaudhari et al., 2009).
Estimating the activities of serum marker enzymes like SGOT and SGPT can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes, normally located in cytosol, are released into the bloodstream. Their estimation in the serum is a useful quantitative market of the extent and type of hepatocellular damage (Reitman et al., 1998).

Serum glutamic oxaloacetic transaminase (SGOT) is one of the liver enzymes and it is also known as Aspartate transaminase (AST). It catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and is an important enzyme in amino acid metabolism. AST is a protein made by liver cells. When liver cells are damaged, AST leaks out into the bloodstream and the level of AST in the blood becomes higher than normal. AST is different from ALT because AST is found in parts of the body other than the liver including the heart, kidneys, muscles and brain. When cells in any of those parts of the body are damaged, AST will be elevated. The enzyme AST was found to be increased in ethanol intoxicated rats which indicates some damage in liver cells. In contrast, *Osheckia oclandra* aqueous extract reduced the AST in ethanol intoxicated rats (Jayaweera, 1982). Similarly, 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). In the reverse reaction, aspartate may be synthesized from oxaloacetate, which is a key intermediate in the citric acid cycle (Berg et al., 2006). In the present study, the
administration of herbal drugs *A. marmelos* and *E. prostrata* also showed a decreased level of Serum glutamic oxaloacetic transaminase.

Serum glutamic pyruvic transaminase (SGPT) is a transaminase enzyme. It is also called alanine aminotransferase (ALT). It is found in serum and in various bodily tissues, but it is most commonly associated with liver. It catalyzes the two parts of the alanine cycle. It catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being private and glutamate (Ghouri et al., 2010).

Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems (Wang, 2012).

The enzyme ALT was found to be increased in ethanol consumed rats which indicates that there was a significant damage in liver cells. But ethanolic extracts of *Eclipta alba* decreased the synthesis of ALT regulating the levels of hepatic microsomal drug metabolizing enzymes (Saxena et al., 1993). The earlier studies strongly agree with the present study. In the present study, the administration of herbal drugs *A. marmelos* and *E. prostrata* also showed decreased amount of serum glutamic pyruvic transaminase. Among the drugs, drug from *A. marmelos*
was more effective in decreasing the level of ALT than that of *E. prostrata*.

Gamma-glutamyl transferase (GGT) is found in many tissues, the most notable one being the liver and has significance in medicine as a diagnostic marker. GGT catalyzes the transfer of the gamma glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione, drug and xenobiotic detoxification (Siest *et al.*, 1992).

Alcohol increased the GGT production by inducing hepatic microsomal production which causes the leakage of GGT from hepatocytes (Barouki *et al.*, 1983). The ethanol extract of the leaves of *Ziziphus mauritiana* decreased the GGT production in ethanol fed albino rats (Dahiru *et al.*, 2005). Likewise, the Nigerian plant, *Alchornea cordifolia* also steps down the GGT synthesis (Olaleye *et al.*, 2006). Thus in the present study *A. marmelos* effectively decreased the amount of GGT than *E. prostrata*.

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as a basic phosphatase (Tamás *et al.*, 1983).
Alkaline phosphatase is an enzyme made in liver cells, bile ducts, kidney, bone and the placenta. A high alkaline phosphatase level occurs when there is a blockage of flow in the biliary tract or a build up of pressure in the liver often caused by a gallstone in the bile ducts.

Gupta et al. (2006) reported that the ethanol extract of the leaves of *Chamomilerrecutita capitula* decreased the ALP production in ethanol fed albino rats (Dahiru et al., 2005). Similarly, aqueous extract of *Rhoicissus tridentate* aqueous extract reduced the level of ALP production in CCl₄ intoxicated albino rats. In the present study, the administration of herbal drugs from *A. marmelos* and *E. prostrata* decreased the synthesis of ALP. The effect was more with *A. marmelos* than with *E. prostrata*.

Bilirubin is a yellowish substance that is created by the breakdown or destruction of haemoglobin, a major component of red blood cells. Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment that is also a product of heme catabolism (Stocker et al., 1987). Bilirubin is released from the destroyed red blood cells and passed on to the liver. The liver excretes the bilirubin in the fluid called bile. If the liver is not functioning properly, the bilirubin will not be properly excreted. Therefore, if the bilirubin level is higher than normal, it may mean that the liver is not functioning correctly (Baranano et al., 2002). The methanolic extract of the leaves of *Ficus carica* decreased the synthesis of total bilirubin regulating the levels of hepatic microsomal drug metabolizing enzymes (Krishna et al., 2007).
Similar findings were also reported by other workers (Singanan et al., 2007) which was done with the methanolic extract Andrographis paniculata. In the present study, the administration of herbal drugs A. marmelos and E. prostrata decreased the synthesis of bilirubin and A. marmelos effectively diminished the production of bilirubin in the ethanol intoxicated albino rats as compared to E. prostrata.

Serum total protein also called plasma total protein is made up of albumin and globulin. Albumin helps to prevent fluid from leaking out of blood vessels. Globulins are the important parts of our immune system. High levels of serum total protein are seen in patients with liver disease, multiple myeloma, rheumatoid arthritis and chronic infections, alcoholism, leukemia and tuberculosis. An elevated level of serum total protein was observed in ethanol consumed albino rats. But in contrast, Vadivel et al. (2008) reported that alcoholic extract of the fruits of Coccinia grandis decreased the serum total protein in alcohol consumed albino rats. Likewise, ethanol extract of Sargassum polycystum also decreased the serum total protein in alcohol consumed albino rats (Meena et al., 2008). In the present study, the administration of herbal drugs A. marmelos and E. prostrata decreased the synthesis of serum protein and A. marmelos effectively stepping down the production of serum protein in the ethanol intoxicated albino rats.

From the foregoing discussion, it is concluded that the leaf extracts of A. marmelos and E. prostrata could be a potential new natural source of medicine for liver disorders. However, the extract of A. marmelos
showed potential hepatoprotective activity than *E. prostrata*. Further studies are needed to reveal the exact mechanism of action responsible for hepatoprotective activities.

5.5. HISTOPATHOLOGICAL ANALYSIS

In histopathological studies, sheets of connective tissues divide the liver into thousands of small units called lobules. A lobule is roughly hexagonal in shape, with portal triads at the verticals and a central vein in the middle. The lobule is the structural unit of the liver which was easy to observe. In contrast, the hepatic acinus is more difficult to visualize, but represents a unit that is of more relevance to hepatic function because it is oriented around the afferent vascular system (Bishayee *et al.* 1995). The parenchymal cells of the liver are hepatocytes. These polygonal cells are joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes (Raj *et al.*, 2009).

The appearance of ultrastructure of hepatocytes reflects their function as metabolic superstars, with abundant rough and smooth endoplasmic reticulum and Golgi membranes. Glycogen granules and vesicles containing very low density lipoproteins are readily observed. Hepatocytes make contacts with blood in sinusoids, which are distensible vascular channels lined with highly fenestrated endothelial cells and populated with phagocytic Kupffer cells (Arulkumaran *et al.*, 2009). The space between endothelium and hepatocytes is called the Space of Disc which collects lymph for delivery to lymphatic capillaries. Bile
originates as secretions from the basal surface of hepatocytes, which collects in channels called canaliculi.

These secretions flow toward the periphery of lobules and into bile duct and interlobular bile ducts, ultimately collecting in the hepatic duct outside the liver. The hepatic duct is continuous with the common bile duct, which delivers bile into duodenum. In most species, bile is diverted through cystic duct into the gall bladder. The columnar epithelium of the gall bladder is devoted largely to the absorption of water and electrolytes (Huo et al., 2011).

In the present histological studies, hepatocytes of the normal group showed a normal lobular architecture of the liver. Whereas, in the alcohol treated group, the liver showed hepatocytic necrosis and inflammation was also observed in the centrilobular region with portal triaditis. Intralobular veins were damaged but to a lesser extent. Endothelium is disrupted and hepatic cells adjoining to intralobular vein show atrophy. In the present investigation, sections of the liver treated with extracts of leaves of both the plants and alcohol, reveals better hepatoprotective activity. In another study, it has been reported that the aqueous extracts of Annona squamosa treated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal in alcohol induced albino rats (Saleem et al., 2008). Similar results have been reported in methanol, hexane and chloroform extracts of Prostecheam ichuacana against CCl₄ induced hepatic injury in albino rats (Rosa and Rosario, 2009).
The test plants *A. marmelos* and *E. prostrata* treated groups showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. Almost negligible damage to a few hepatocytes present in the close vicinity of intralobular veins was observed in test plants. Endothelium lining is almost smooth except in one or two places. Hepatocytes exhibited normal appearance only in some cells which have a higher number of vacuoles in the cytoplasm. Silymarin treated group showed normal hepatocytes and their lobular architecture was normal. The results of histopathological study also support the results of biochemical parameters.