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# EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *LEUCAS CEPHALOTES* IN INDUCED LIVER INJURY IN RATS

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**Abstract:**

*In this study we investigated the in vivo Hepatoprotective activity of ethanolic extract of Leucas cephalotes in induced hepatotoxicity using Wistar rats of either sex as model. Hepatotoxicity was induced by the administration of 3% CMC per 100g body weight). Leucas cephalotes extract at different dose levels (200 and 400mg/kg, p.o.) showed the dose dependent hepatoprotective effect and was compared with well-known standard hepatoprotective Silymarain (100mg/kg).*

*The rats were divided into five groups with three rats in each for three models. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 21 days. Group II (control and 40% ethanol) animals on the 21 day. Group III Received 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days and standard drug silymarin (25 mg/kg. p.o.) for 21 days once daily and IV were treated with 40% ethanol and Ethanolic extract Leucas cephalotes (400 mg/kg) 21 days once daily. Group V was treated with Received 40% ethanol and Ethanolic extract of Leucas cephalotes (200 mg/kg) 21 days once daily the animals were sacrificed 48 h after the last injection of hepatotoxic drugs under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters.*

*In hepatoprotective studies, the toxicity elevated levels of serum marker enzymes Total Bilirubin ALT, AST, ALP, SGOP and SGPT levels. Ethanol induced hepatotoxicity was significantly prevented by pretreatment ethanolic extract of leaves. Decrease in wet liver weight, reduction in elevated biochemical parameter levels like serum SGPT, SGOT, and total bilirubin, after treatment with Ethanolic extract of Leucas cephalotes confirmed the hepatoprotective effect of extract under study. In liver injury models in rats' restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection.*

*The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant at $p < 0.01$ for ANOVA and $P < 0.05$ for t-test.*

***Keywords:*** *Leucas cephalotes, Hepatoprotective activity, Alkaline Phosphate, Aspartate amino trasferase, Alanine amino transferase.*

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## INTRODUCTION

1.1 LIVER

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles [1].

Anatomical Position

The liver is predominantly located in the right hypochondrium and epigastric areas, and extends into the left hypochondrium.

When discussing the anatomical position of the liver, it is useful to consider its external surfaces, associated ligaments, and the anatomical spaces (recesses) that surround it.

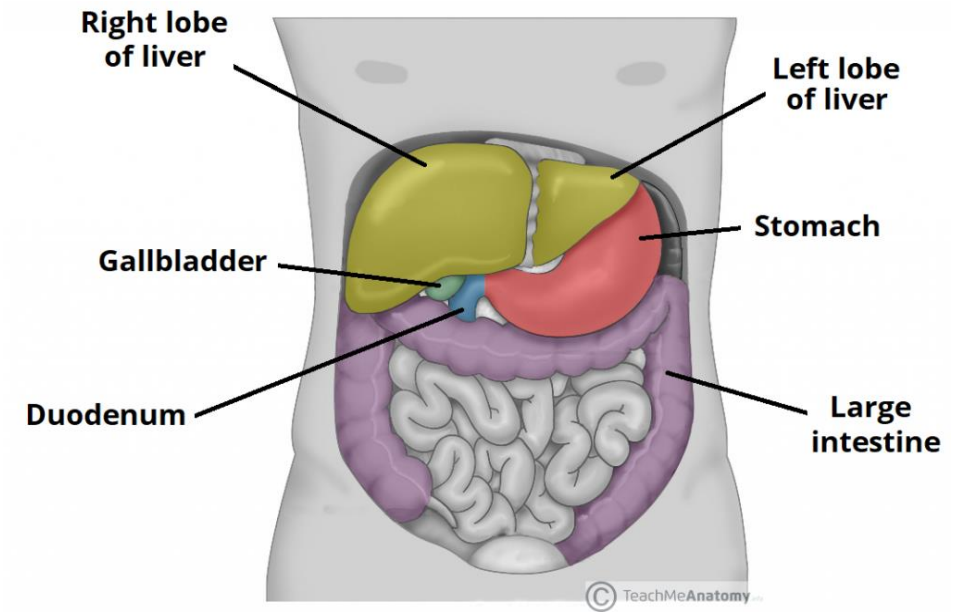


Figure : 1: Anatomical position of liver

Liver Surfaces

The external surfaces of the liver are described by their location and adjacent structures. There are two liver surfaces – the diaphragmatic and visceral:

- Diaphragmatic surface – the anterosuperior surface of the liver.
  - It is smooth and convex, fitting snugly beneath the curvature of the diaphragm.
  - The posterior aspect of the diaphragmatic surface is not covered by visceral peritoneum, and is in direct contact with the diaphragm itself (known as the 'bare area' of the liver).

- Visceral surface – the posteroinferior surface of the liver.
  - With the exception of the fossa of the gallbladder and porta hepatis, it is covered with peritoneum.
  - It is moulded by the shape of the surrounding organs, making it irregular and flat.
  - It lies in contact with the right kidney, right adrenal gland, right colic flexure, transverse colon, first part of the duodenum, gallbladder, oesophagus and the stomach.

Ligaments of the Liver

There are various ligaments that attach the liver to the surrounding structures. These are formed by a double layer of peritoneum.

- Falciform ligament – this sickle-shaped ligament attaches the anterior surface of the liver to the anterior abdominal wall and forms a natural anatomical division between the left and right lobes of the liver. The free edge of this ligament contains the ligamentum teres, a remnant of the umbilical vein.

- Coronary ligament (anterior and posterior folds) – attaches the superior surface of the liver to the inferior surface of the diaphragm and demarcates the bare area of the liver The anterior and posterior folds unite to form the triangular ligaments on the right and left lobes of the liver.

- Triangular ligaments (left and right):
  - The left triangular ligament is formed by the union of the anterior and posterior layers of the coronary ligament at the apex of the liver and attaches the left lobe of the liver to the diaphragm.

- The right triangular ligament is formed in a similar fashion adjacent to the bare area and attaches the right lobe of the liver to the diaphragm.
- Lesser omentum – Attaches the liver to the lesser curvature of the stomach and first part of the duodenum. It consists of the hepatoduodenal ligament (extends from the duodenum to the liver) and the hepatogastric ligament (extends from the stomach to the liver). The hepatoduodenal ligament surrounds the portal triad.

In addition to these supporting ligaments, the posterior surface of the liver is secured to the inferior vena cava by hepatic veins and fibrous tissue.

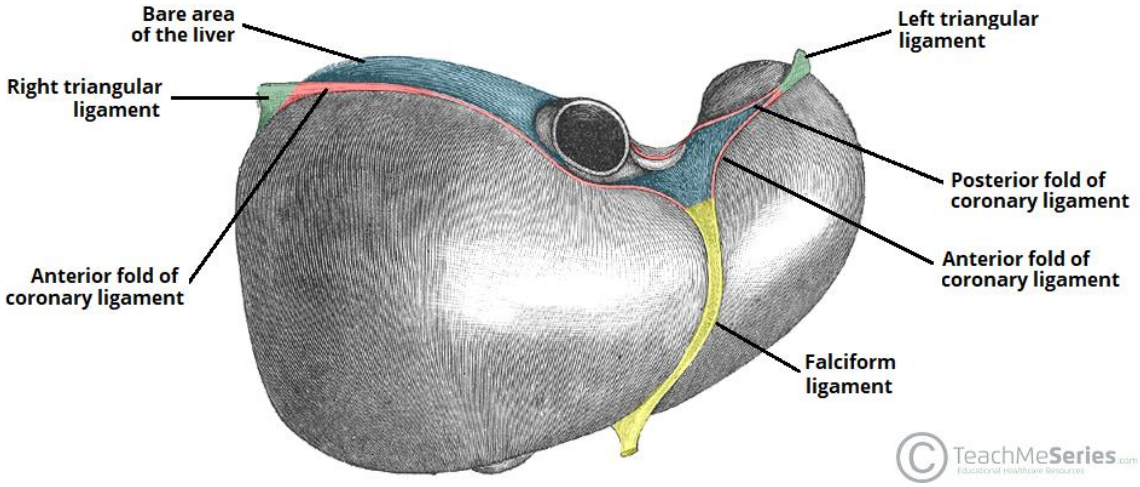


Fig 2 – Diaphragmatic surface of the liver, demonstrating the three main ligaments. The bare area of the liver lies between the anterior and posterior folds of the coronary ligament.

Hepatic Recesses

The hepatic recesses are anatomical spaces between the liver and surrounding structures. They are of clinical importance as infection may collect in these areas, forming an abscess.

- Subphrenic spaces – located between the diaphragm and the anterior and superior aspects of the liver. They are divided into a right and left by the falciform ligament.
- Subhepatic space – a subdivision of the supracolic compartment (above the transverse mesocolon), this peritoneal space is located between the inferior surface of the liver and the transverse colon.
- Morison's pouch – a potential space between the visceral surface of the liver and the right kidney. This is the deepest part of the peritoneal cavity when supine (lying flat), therefore pathological abdominal fluid such as blood or ascites is most likely to collect in this region in a bedridden patient.

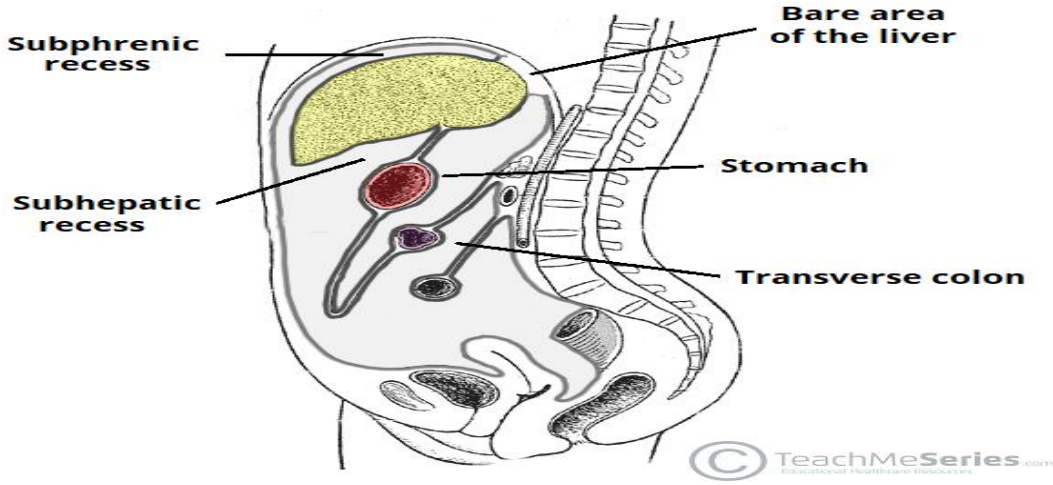


Fig 3 – The subphrenic and subhepatic recesses.

Anatomical Structure

The structure of the liver can be considered both macroscopically and microscopically.

Macroscopic

The liver is covered by a fibrous layer, known as Glisson's capsule.

It is divided into a right lobe and left lobe by the attachment of the falciform ligament. There are two further 'accessory' lobes that arise from the right lobe, and are located on the visceral surface of liver:

- Caudate lobe – located on the upper aspect of the visceral surface. It lies between the inferior vena cava and a fossa produced by the ligamentum venosum (a remnant of the fetal ductus venosus).
- Quadrate lobe – located on the lower aspect of the visceral surface. It lies between the gallbladder and a fossa produced by the ligamentum teres (a remnant of the fetal umbilical vein).

Separating the caudate and quadrate lobes is a deep, transverse fissure – known as the porta hepatis. It transmits all the vessels, nerves and ducts entering or leaving the liver with the exception of the hepatic veins.

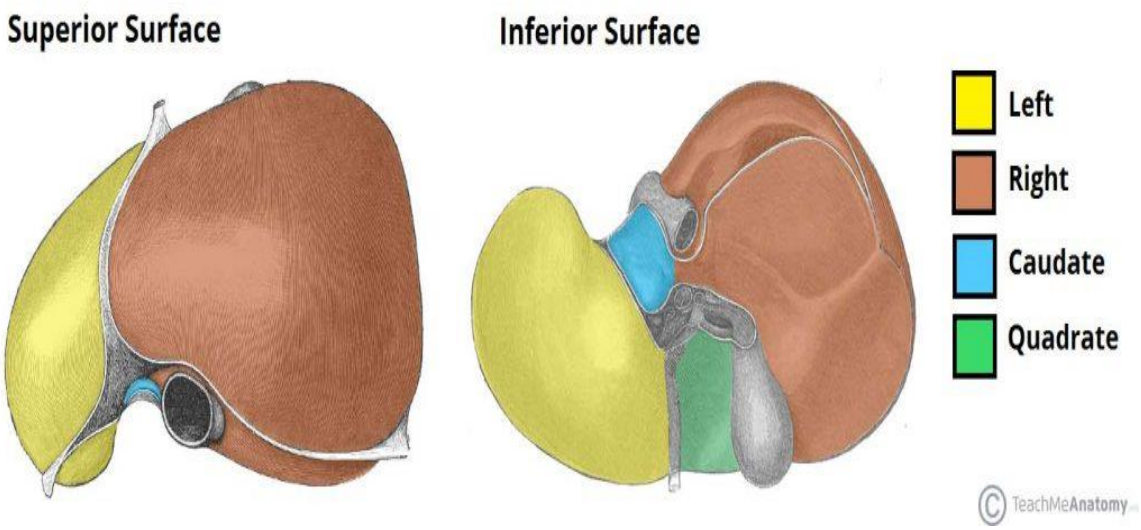


Fig 4 – The anatomical lobes of the liver.

Microscopically, the cells of the liver (known as hepatocytes) are arranged into lobules. These are the structural units of the liver.

Each anatomical lobule is hexagonal-shaped and is drained by a central vein. At the periphery of the hexagon are three structures collectively known as the portal triad:

- Arteriole – a branch of the hepatic artery entering the liver.
- Venule – a branch of the hepatic portal vein entering the liver.
- Bile duct – branch of the bile duct leaving the liver.

The portal triad also contains lymphatic vessels and vagus nerve (parasympathetic) fibres.

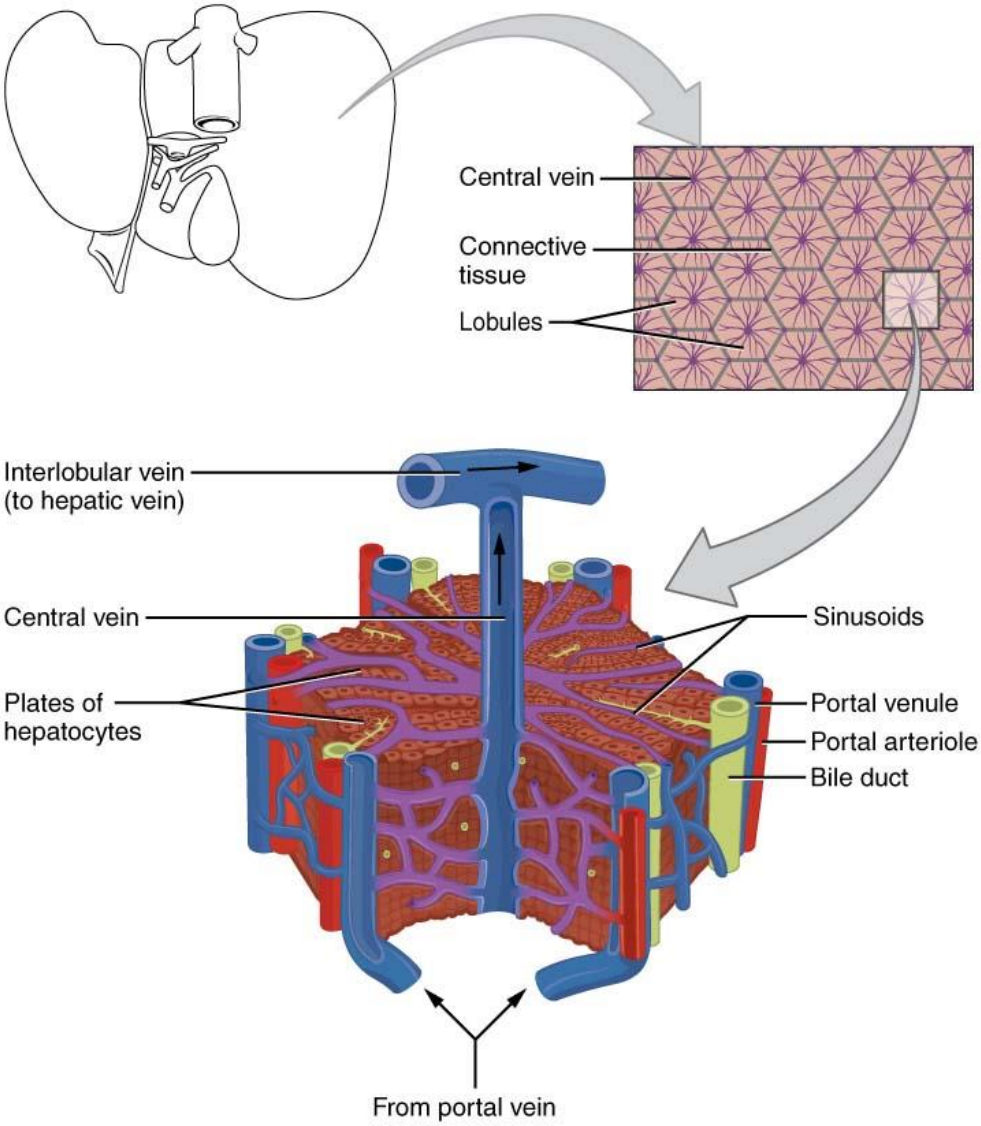


Fig 5 – The structures of a hepatic lobule.

Arterial Supply and Venous Drainage

The liver has a unique dual blood supply:

- Hepatic artery proper (25%) – supplies the non-parenchymal structures of the liver with arterial blood. It is derived from the coeliac trunk.
- Hepatic portal vein (75%) – supplies the liver with partially deoxygenated blood, carrying nutrients absorbed from the small intestine. This is the dominant blood supply to the liver parenchyma, and allows the liver to perform its gut-related functions, such as detoxification.

Venous drainage of the liver is achieved through hepatic veins. The central veins of the hepatic lobule form collecting veins which then combine to form multiple hepatic veins. These hepatic veins then open into the inferior vena cava.

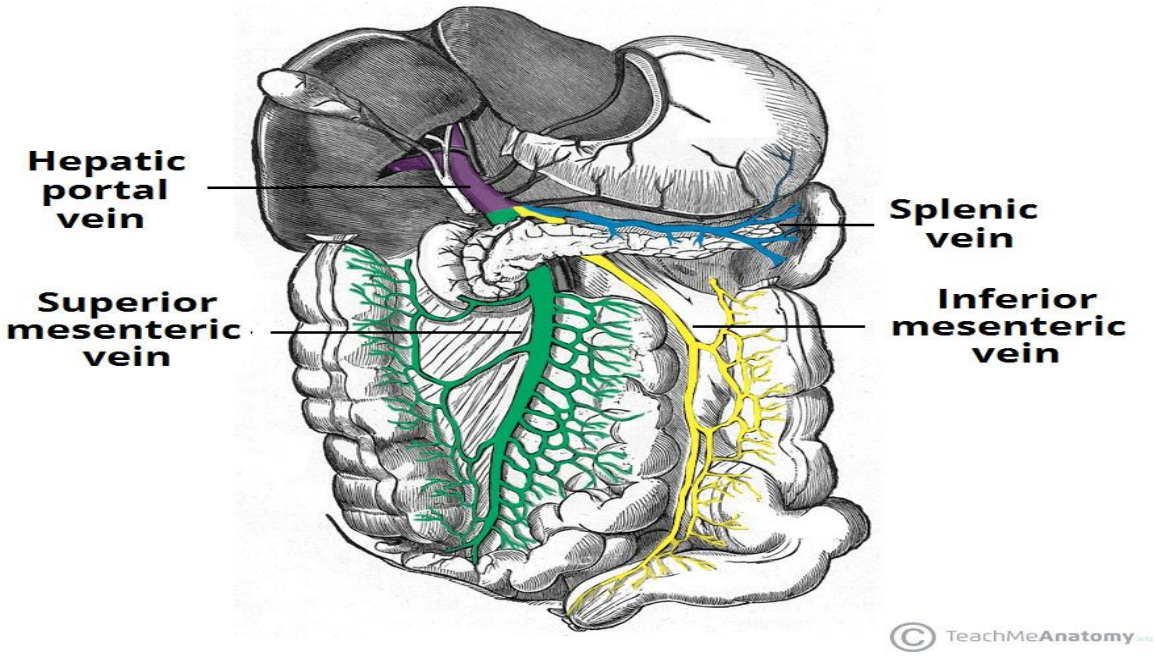


Fig 6 – An overview of the venous portal system – draining into the hepatic portal vein.

Nerve Supply

The parenchyma of the liver is innervated by the hepatic plexus, which contains sympathetic (coeliac plexus) and parasympathetic (vagus nerve) nerve fibres. These fibres enter the liver at the porta hepatis and follow the course of branches of the hepatic artery and portal vein.
Glisson's capsule, the fibrous covering of the liver, is innervated by branches of the lower intercostal nerves. Distension of the capsule results in a sharp, well localised pain.

Lymphatic Drainage

The lymphatic vessels of the anterior aspect of the liver drain into hepatic lymph nodes. These lie along the hepatic vessels and ducts in the lesser omentum, and empty in the colic lymph nodes which in turn, drain into the cisterna chyli.
Lymphatics from the posterior aspect of the liver drain into phrenic and posterior mediastinal nodes, which join the right lymphatic and thoracic ducts.

Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them[2].

Liver cells possess the antioxidant defence system consisting of antioxidants such as GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death [3].

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic [4]. Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances [5]. During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which cause oxidative stress [6] which leads to the hepatic damage.

1.1.1. Liver diseases

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease; drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of

the ten most common tumors in the world with over 2,50,000 new cases each year[7].

Infections

Sometimes, the problem is that you have an infection that inflames your liver. Viral hepatitis is the most common cause, including:

- Hepatitis A. Most people get it by eating or drinking something that's tainted by fecal matter. You might not have any symptoms. It usually goes away by itself within 6 months without any long-term harm.
- Hepatitis B. You get it from somebody else, such as through unprotected sex or taking drugs with shared needles. If it lasts longer than 6 months, it makes you more likely to get liver cancer or other diseases.
- Hepatitis C comes from infected blood that gets into your blood. You might get it if you take drugs with shared needles or in connection with HIV. If you're a health-care worker, you might get it from an infected needle that accidentally sticks you. Symptoms may not show up for many years. For reasons that aren't quite clear, baby boomers are at risk for hepatitis C and should be tested for it.

Immune System Problems

Your immune system fights off invaders including bacteria and viruses. But it might go wrong and attack one or more parts of your body, such as your liver.

- Autoimmune hepatitis inflames your liver. It can lead to other disorders and even liver failure. It strikes girls and women more often than boys or men.
- Primary biliary cholangitis attacks tiny tubes in your liver called bile ducts. They carry bile, a chemical that helps you digest food. When the ducts are injured, the bile backs up inside your liver and scars it. Women come down with this more often than men.
- Primary sclerosing cholangitis scars your bile ducts, and it can eventually block them. The bile builds up inside your liver, and that makes it harder for your liver to work. It may lead to liver cancer, and you might someday need a liver transplant. Men are more likely than women to get it.

Cancer and Tumors

If cancer shows up in your liver, that's most likely because it has spread from another part of your body, like your lungs, colon, or breasts. But a few cancers can start in the liver.

- Liver cancer affects women more often than men, and African-Americans more often than whites. Your doctor might call it hepatocellular carcinoma. It's more likely if you have hepatitis or drink too much.
- Bile duct cancer strikes the tubes that run from your liver to your small intestine to carry bile, a fluid that helps you digest food. This kind of cancer mainly affects people over age 50, but it's uncommon.
- Liver cell adenoma is a tumor that doesn't have cancer. It's uncommon, but women who take birth control pills for a long time are more prone than other people to develop it. There's a small chance the tumor could eventually turn into cancer.

Conditions You Inherit

Some inherited liver disorders only happen if they run in your family.

- Hemochromatosis makes your body store up too much of the iron from your food. The extra iron builds up in your liver, heart, or other organs. It can lead to life-threatening conditions such as liver diseases, heart disease, or diabetes.
- Hyperoxaluria hits when your urine has too much of a chemical called oxalate. In this condition, your liver makes too little much oxalate due to a genetic mutation. This can cause kidney stones and kidney failure. If your kidneys do fail, that can give you oxalosis, where the oxalate collects in other organs and causes more trouble.
- Wilson's disease makes copper build up in your liver and other organs. Its first symptoms usually show up when you're between the ages of 6 and 35, most often in your teens. It not only affects your liver, but it can cause nerve and psychiatric problems.
- Alpha-1 antitrypsin deficiency involves a chemical that helps your lungs resist infections. Your liver makes it. But when your liver gets the recipe wrong, the faulty chemical can build up and cause liver disease.

Other Causes of Liver Disease

- Alcohol abuse can lead to cirrhosis. So can nonalcoholic fatty liver disease and long-term cases of hepatitis B and C.

- Drug overdoses. Taking too much acetaminophen or other medications can harm your liver. Make sure you follow the dosing instructions on the label, and be aware that acetaminophen might be in more than one medicine you take.
- Nonalcoholic fatty liver disease (NAFLD) is when too much fat has built up inside your liver. The extra fat can inflame your liver. One type of NAFLD is nonalcoholic steatohepatitis (NASH). It means you have inflammation and cell damage in your liver, as well as fat. It can scar your liver and lead to other disorders, like cirrhosis.

Dire complications of liver disease include:

- Acute liver failure. This happens when you don't have a long-term liver disease but your liver quits working within a very short time -- days or weeks. That may happen because of an overdose of acetaminophen, infections, or because of prescription drugs.
- Cirrhosisis a buildup of scars in your liver. The more scars replace the healthy parts of your liver, the harder it is for your liver to do its job. Over time, it may not work like it should.

According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually[8].

Depending on the duration of the disease the liver diseases are classified as acute or chronic. If the disease does not exceed six months it is considered as acute liver disorder while diseases of longer duration are classified as chronic. Acute viral hepatitis and drug reactions account for the majority of cases of acute liver disease. Hepatitis A and B are the commonest causes of viral hepatitis in Europe and hepatitis E is common in India. Hepatitis C is not usually recognised as an acute infection because it rarely causes jaundice at this stage.

Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer[9]. Chronic hepatitis or long term intoxification can severely injure hepatic cells. Initially, the damaged cells are denatured, but subsequently transformed to hypertrophic fibrosis and necrosis, and eventually may progress to hepatoma.

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, schistosomiasis, etc; or chemical factors such as $CCl_4$ administration, alcohol intake, etc. were known to contribute to liver fibrosis. Hepatic fibrosis is major features of a wide range of chronic liver injuries including metabolic, viral, cholestatic and genetic disease. The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes and causes apoptosis and/or necrosis [10].

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis C [11&12]. In many patients, hepatitis such as non-alcoholic fatty liver disease becomes chronic and eventually progresses to more serious liver pathologies, such as fibrosis, cirrhosis, or even carcinogenesis, causing devastating economic losses and mortality[13].

Drug/chemical-mediated hepatic injury is the common sign of drug toxicity[14] and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for withdrawal of drugs from the market [15]. Drug-induced liver disease can be predictable (high incidence and dose-related) or unpredictable (low incidence and may or may not be dose-related). Unpredictable reactions, also referred to as idiosyncratic, can be viewed as either immune-mediated hypersensitivity or nonimmune reactions. Most potent predictable hepatotoxins are recognized in the animal testing or clinical phase of drug development.

1.1.2. Drugs for liver diseases

The liver is quantitatively the most important site of drug metabolism. However, many drugs are known to cause hepatic injury. Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects.

Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically. Current medical treatments for these liver diseases are often ineffective, and therefore efforts are being made to seek new effective medications[16]. Developing pharmacologically effective agents from natural products has become a new trend by virtue of their

little toxicity or few side effects. There are few plant derived drugs in the market which are used for the liver disorders.

- Silymarin

Silymarin, derived from the seeds of Silybum marianum L. (Family: Asteraceae or Compositae), commonly known as milk thistle, has been used for centuries as a natural remedy for liver and biliary tract diseases[17]. Milk thistle protects and regenerates the liver in most liver diseases such as cirrhosis, jaundice, and hepatitis[18]. Silymarin offers good protection in various models of experimental liver disease. It acts by antioxidative, antilipid peroxidative[19], antifibrotic[20], membrane stabilizing, immunomodulatory and liver regenerating mechanisms [21].

**Limitations**

Silymarin is insoluble in water and typically administered as a sugar coated tablet or as an encapsulated standardized extract. The absorption by oral route is as low as 2-3 percent of the silybin recovered from rat bile in 24 h. About 20-40 percent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings.

Side Effects

Silymarin has low level of toxicity. Although, silymarin has a good safety record, there are few reports of gastrointestinal disturbances and allergic skin rashes.

- Liv-52

Liv-52 was introduced in 1954 as a specially formulated Ayurvedic herbal remedy for the treatment of viral hepatitis and has been widely prescribed for infective hepatitis since then[22]. Experimentally, Liv-52 prevented injurious effects of carbon tetrachloride and other toxic substances on the liver. Liv.52 is available as tablets and syrup containing the following herbs: Capparis spinosa; Cichorium intybus; Solanum nigrum; Terminalia arjuna; Cassia occidentalis, Achillea millefolium; Tamarix galica and Phyllanthus amarus. These herbs are processed and formulated according to the principles of Ayurveda, which are aimed at enhancing efficacy and avoiding toxicity[23].

Drug-induced hepatotoxicity:

Hepatotoxicity is defined as injury or liver damage caused by exposure to drugs or other nonpharmacological agents [1]. It is an adverse drug reaction that may be uncommon but serious, and therefore, have a considerable impact on health [2]. Hepatotoxicity generates between 1/600 and 1/3500 of all hospital admissions, 2–3% of hospitalizations for jaundice, 10% of acute jaundice hepatitis (being more than 40% in people over 50 years of age) and between 15 and 30% of cases of fulminant hepatic failure [15, 16]. In the United States, a multicenter prospective study showed that drugs, including acetaminophen, are the most common cause of acute liver failure, explaining 39% of cases and overcoming viral hepatitis A and B, which represent 12% [17]. On the other hand, in France, an incidence of 13.9 (±2.4) cases per 100,000 inhabitants is estimated, corresponding to an annual global frequency of 8.1 (±1.5) cases [18]. In Switzerland, the estimated incidence is 2.2 per 100,000 inhabitants over 15 years of age; while in Spain, the annual incidence of severe liver disease is estimated as 7.4 per 1,000,000 population (95% confidence interval between 6.0 and 8.8) [10]. There are some countries in which evidence about incidence of liver injury by drugs is limited [19], generally, published information is based on clinical case reports.

Although hepatotoxicity is less frequent than other adverse drug effects, due to its severity and is the most common cause of drug withdrawal in the pharmaceutical market, it is assessed as a major adverse event [20], so, it is a frequent impediment to the development of drugs by pharmaceutical companies. In the past 20 years, in Europe and the United States medications such as troglitazone, bromfenac, trovafloxacin, ebrotidine, nimesulide, nefazodone, ximelagatran, lumiracoxib, pemoline and tolcapone have been withdrawn from the market [10, 21, 22, 23]; currently, some of them are retired worldwide.

The identification of hepatotoxicity is a complex process to perform; therefore, in clinical practice, it is based on considering the presence of such alteration, investigate about the use of any substance and ruling out other causes of liver disease [19]. It is necessary to identify all drugs used (prescription and over-the-counter), natural products, food, exposure to industrial toxics or substance abuse; moreover, try to identify the offending agent and to search for a description in literature may help. Besides, the chronological relationship between exposure to the suspected agent and the hepatotoxic reaction is a key to define causality; the drugs used in the last 3 months should be considered as suspects. The presence of hypersensitivity manifestations (rash, fever and eosinophilia) improves identification process as well as histological analysis through liver biopsy [24]. When a re-exposure to the suspected

agent appears, it becomes a very conclusive indicator of causality.

There is no specific treatment for hepatic toxicity by drugs, which is based on suspending the suspected drug, treating symptoms (use of corticosteroids for hypersensitivity reactions), avoiding other possible hepatotoxic agents and continuous monitoring of laboratory tests [13, 14

There are some exceptions of antidotes for treating liver toxicity by certain drugs such as the use of N-acetyl cysteine as an antidote for acetaminophen toxicity, or N-acetyl cysteine itself for the treatment of hepatotoxicity by phenytoin and carbamazepine, or carnitine for valproic acid toxicity [25]. With the suspension of the offending drug, in most cases, the health of the patients tends to improve; however, in other cases, the damage continues to progress and hospitalization is necessary, when irreversible liver failure occurs, liver transplantation is required and if the liver tissue damage is severe, patients can die in a few hours.

Hepatotoxicity associated to drugs

Hepatotoxicity induced by drugs or toxins can be grouped into two types: intrinsic reactions (less common) and idiosyncratic reactions (more common) [6, 7, 26]. Intrinsic reactions are predictable, dose-dependent and reproducible in animal models; injury is produced trough toxic metabolites of drugs such as free radicals (generating lipid peroxidation), electrophilic molecules (formation of covalent bonds with hepatic proteins) or active oxygen molecules (generating peroxidation as well). Idiosyncratic reactions are not predictable, not dose-dependent and not reproducible in animals; there are many drugs capable of causing this type of reaction [11, 27]. The underlying mechanism of the idiosyncratic reaction may be a genetic polymorphism of the cytochrome P450 (CYP450) system, responsible for the drugs hepatic biotransformation. There are two types of idiosyncratic reactions: immune (characterized by hypersensitivity-type reaction) and metabolic [7, 13] (related to metabolism of substances).

Mechanisms of hepatotoxicity

The hepatocytes, cholangiocytes, Kupffer cells, ductal and endothelial cells are involved in the mechanisms by which drugs cause hepatotoxicity [28]; having direct effects on cellular organelles such as mitochondria, endoplasmic reticulum, cytoskeleton, microtubules or nucleus.

The drug metabolites generated in the liver through biotransformation can cause hepatic damage because formation of toxic or reactive substances such as electrophilic chemicals or free radicals [29], and thus an unchain a variety of chemical reactions may happen. These mechanisms can either generate necrosis or apoptosis or both. The following are some of the main mechanisms of liver injury [30]:

Mitochondrial dysfunction: may be generated by the disruption

## MATERIALS AND METHODS

### Chemicals

Ethanol Absolute
Petroleum ether
Silymarin
SGOT enzyme Kit (Giri diagnostic kit pvt ltd)
SGPT enzyme kit (Giri diagnostic kit pvt ltd)
Chloroform (Central drug house pvt ltd) New Delhi

## PHYTOCHEMICAL ANALYSIS

Preliminary chemical tests were carried out for methanolic extract to identify different Phyto-constituents.

### 3.1. Alkaloids

The crude powder and methanol extract of *Leucas cephalotes* was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3equal portions. One portion was treated with few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicated the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was recorded if heavy precipitate or flocculation was observed.

### 3.2. Flavonoids

#### 3.2.1. Shinoda test

The presence of flavonoids was estimated by Shinoda test. The crude powder and methanol extract of *Leucas cephalotes* were treated with few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red colour within few minutes indicated the presence of flavonoids.

#### 3.2.2. Alkaline reagent test

The crude powder and methanol extract of *Leucas cephalotes* was treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.

**3.3. Cardiac glycosides**

Keller-kiliani test was performed for the presence of cardiac glycosides. The crude powder and methanol extract of *Leucas cephalotes* was treated with 1 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few drops of concentrated $H_2SO_4$ were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides.

**3.4. Plobotannins**

The crude powder and methanol extract of *Leucas cephalotes* was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatanins.

**3.5. Saponins**

The presence of saponins was determined by Frothing test. The crude powder and methanol extract of *Leucas cephalotes* was vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5cm indicated the presence of saponins.

**3.6. Steroids**

Liebermann-Burchard reaction was performed for the presence of steroids. Achloroformic solution of the crude powder and methanol extract of *Leucas cephalotes* was treated with acetic anhydride and few drops of concentrated $H_2SO_4$ were added down the sides of test tube. A blue green ring indicated the presence of steroids.

**3.7. Tannins**

The crude powder and methanol extract of *Leucas cephalotes* was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue color indicated the presence of tannins.

**3.8. Triterpenes**

Chloroform extract of the crude powder and methanol extract of *Leucas cephalotes* was treated with concentrated sulphuric acid ($H_2SO_4$). Appearance of reddish brown ring indicated the presence of triterpenes.

**Mechanism of Action of silymarin**

The mechanisms which provide silymarin hepatoprotective effects are many and varied, and include antioxidation, anti-lipid peroxidation, enhanced detoxification, and protection against glutathione depletion. (Halim AB et al; 1997).

**Stimulation of Liver Regeneration:**

One of the mechanisms to explain the ability of silymarin to stimulate the regeneration of hepatic tissue is the increase in protein synthesis in damaged livers. In both *in vivo* and *in vitro* experiments, significant increases in the formation of ribosome and DNA synthesis were measured in addition to the increase in protein synthesis. Interestingly, the increased protein synthesis was only measured in damaged livers (partial hepectomy), not in controls. The mechanism of increased protein synthesis is currently not known but some authors speculate silymarin imitates a physiologic regulator, so the silybin fits into a specific binding site on the polymerase, thus stimulating ribosome formation. (Schopen RD et al; 1969)

The potential for stimulation of protein synthesis by silymarin was investigated in malignant liver tissue, and no increases in protein synthesis, ribosome formation, or DNA synthesis were found in malignant cell lines. (Sonnenbichler J et al; 1986)

**Alcohol hepatotoxicity**

Alcohol is one of the main causes of end-stage liver disease worldwide In the United States, alcoholic liver disease is the second most common reason for liver transplantation (Mandayalu Jamal et al 2004). The Dionysus Study, a cohort study of the prevalence of chronic liver disease in an Italian population showed that 21% of the population studied was at risk for developing liver damage. Of these, only 5.5 % of the individuals at risk showed actual Signs of liver damage.

About 50 years ago it was believed that alcohol in itself was not toxic, rather that the nutritional deficiencies often accompanying it mere the actual causes of liver damage. However, It was shown by Lieber and De Carli that in rats, alcoholic liver damage developed despite sufficient nutrition (De Carli and Lieber; 1967). The toxicity of alcohol was later on shun to be related to its metabolism by alcohol dehydrogenases (ADHs) and also to the metabolism by CYP2E1. There is also a component of metabolism by catalase (Zilna, Fialova et al. 2001) The main pathway for ethanol (EtOH) oxidation in die liver IS ADH to acetaldehyde, which is associated

with the reduction of NAD to NADH increases in xanthine oxidase activity, which elevates production of superoxide Fialova et al 2001). Metabolism of EtOH by alcohol dehydrogenase Influences the redox status of the liver also in other ways. Enhanced acetaldehyde production after EtOH metabolism decreases hepatic glutathione (GSH) content. The decrease in GSH both due to an increased loss, as well as a lower rate of synthesis (Speisky, MacDonald et al 1985). The absolute majority of EtOH oxidation is by ADH to acetaldehyde and by aldehyde dehydrogenase to acetic acid. However, there is a slight of P450 dependent inducible EtOH oxidation due to the CYP2E1 component. Also, CYP1A2, CYP3A4 and CYP2B families may contribute to EtOH oxidation (Johansson, Ekstrom et al. 1988; Lieber 2004).

The first indication that not only alcohol dehydrogenases participate the metabolism of ethanol came in the early 1970's, when it was discovered that microsomal membrane fractions were capable of catalyzing the oxidation of ethanol. These reactions required NADPH and were inhibited by CO, properties that are distinct from those of alcohol dehydrogenases (Liber. Rubin et al;1970). It was then discovered that this activity was due to CYP2E1 and that the enzyme was inducible by ethanol in rats (Ryan Koop et al. 1986; Johansson Ekstrom et al. 1988)

Free radicals have been implicated in alcoholic liver disease in various ways. Mechanism that are thought to be Involved are impairment of antioxidant defenses, as well as production of reactive oxygen by flie mitochondria and the CYP2E1 enzyme, and by activated phagocytic cells. Oxidative compounds then may lead to activation of immune cells to express pro-fibrotic and pro-Lnflam_matoly cytokines. Macrophages produce TNTa In various conditions that cause oxidative stress (Ahmed, Aronson et al. 2000), as well as IL-1 and IL-6 (Meng and Lowell 1997). Also, oxidative stress leads to the generation of lipid peroxidation products and protein adducts (Ekstrom and Inge1man-Sundberg 1989; Dupont, Lucas et al 1998; Johansson and Inge1man-Sundberg 1985; Albano, French et al 1999), which eventually stimulate a break in self-tolerance and all immune reaction associated with hepatitis (Albano 2006). CYP2E1 Inhibitors have been shown to reduce the formation of lipid peroxidation products (Inge1manSundberg Johansson et al 1993). CYP2E1 has also been should be elevated in non-alcoholic steatohepatltis (Weltman, Farrell et al. 1998). Furthermore it has been shown that the increase m cellular oxidative stress by even moderate alcohol consumption may be one of the mechanisms by which alcoholism progression of chronic hepatitis C, as antibodies towards albumin adducted with lipid peroxidation products were greater in consumers (Riga-monti, Mottaran et al; 2003).

It was suggested that CYP2E1 may play a role in alcoholic liver damage as it has been shovm that during ethanol oxidation CYP22E1 produces O2 -and H202 as a result of uncoupling of oxygen consumption with NADPH oxidation (ingeman-Sundberg and Johansson 1984). In the presence of iron catalysts, even more reactive oxygen species (ROS) can be formed, such as the hydroxyl radical, superoxide and hydrogen peroxide(Ingeman-Sundberg and Johansson 1984; Gonzalez 2005; Cederbaum 2006 and Ingeman-Sundberg 1989); Kou Galeotti et al. 1991). These reactive oxygen species (ROS) may lead to elevated levels of lipid peroxidation products, that in turn adducts with cellular proteins. Adducts may also be formed with nucleic acids. Eventually, this will result In cell damage (Cederbaum 2006) It has been shown that the vanous lipid peroxidation products formed lead to an Immune response in human alcoholics, which worsens as disease progresses (Mot-taraw Stewart et al. 2002). Lipid peroxidation products have also been shown to play a part in fibrosis, by able to activate the stellate cells of die liver to increase their production of collagen which has been particularly studied for the lipid aldehyde malondi aldehyde(MDA) (Maher, Tzagarakis et al. 1994).

Liver injury is often zonated within the liver this is the case for many hepatotoxins that primarily damage the perivenous region and also true in the case of ethanol. Notably, ChQ2E1 is particularly induced in regions by ethanol (Johansson Lindros et al. 1990; Fang, Lindros et al. 1998). vitro studies using HepG2 cells transfected to express CYP2E1, both stably and transiently, have show signs of DNA fragmentation and cell death in transfected cells, which is not seen in controls. EtOH-induced apoptosis was prevented by an Inhibitor of ethanol oxidation Via CP2E1, and also by an antioxidant that prevents lipid peroxidation (wuand Cederbaum 1999) In this cell model it was also noted that glutathione levels were lowered (Wu and Cederbaum 2004)

Although alcohol is undoubtedly a hepatotoxicity still only approximately 20% of heavy drinkers (males) develop alcoholic cirrhosis (Lelbach 1975). Cirrhosis is most often preceded by hepatitis, generally defined by the following morphologic features; hepatocyte necrosis, neutrophil polymorph Infiltrate fatty changes and usually also Mallory bodies

(accumulations of hyaline material in damaged hepatocytes). The series of events preceding cirrhosis are commonly; fatty accumulating hepatitis, fibrosis and finally cirrhosis

Immunological reactions play a large role in alcoholic liver disease. It has long been known that chronic alcoholic liver disease as well as hepatitis is associated with elevated serum cytokine levels, which have prognostic value. TNF alpha is associated with increased mortality in alcoholics (Yin, Wheeler et al. 1999) Serum levels of TNF Alpha receptor 2 and IL-8 have been shown to be significantly elevated in alcoholics (Gonzalez-Reimers, Garcia-Valdecasas-Campelo et al. 2007). Liver disease patients have also been shown to have elevated levels of the proinflammatory cytokines IL-6 and IL-8 (Gonzalez-Quintela, Vidal et al. 1999) as well as TNF alpha and IL-2. IL-6 and IL8 are also elevated m alcoholic patients without liver disease.

Interestingly, also levels of anti-inflammatory cytokines such as IL-IO are elevated in alcoholics with liver disease cirrhosis (Latvala& Hietala et al. 2005; Szuster-Ciesielska Damluk et al. 2000). Clinically, dietary supplementation with SAM to restore glutathione levels, and phosphatidylcholines to restore membrane finction have been successful and proved some favorable effects on parameters of liver damage (Liebr 2005). S-Adenosy1 methionine (SAM) is a precursor of glutathione. The drug N-acetylcysteine NC), also a glutathione precursor, is used clinically to prevent hepatic failure in the case of paracetamol overdose (Lauterburg, Corcoran et al. 1983; Shah and Gordon 2007). The beneficial effect of SAM has also been shown in vanous animal models. In rats exposed to ethanol and LPS, SABA treated animals showed significantly decreased fibrosis, oxidative stress and steatosis. Supplementation also improves liver function SAM also decreases as TGFß mRNA levels and stellate cell activation (Kapaa 2008) SAM has also been proved to attenuate EtOH induced glutathione depletion and associated mitochondrial lesions m baboons (Lieber 1994).

**Animals**

Wistar albino rats of both sexes (180-220 g) were used for the study. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house of Department of Pharmacology. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Baroda. Amruth Brand rat & mice pellet feed) and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

**Acute toxicity studies:**

Healthy Wistar albino female rats of sex weighing 100-170 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Co-operation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h. Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes.

**Determination of acute toxicity (LD50):**

14 days single dose oral acute toxicity and gross behavioral study Number of animals required: 6 rats (male) Number of groups: 2 groups (3 animals each group).

Dose levels: 4000 mg/kg body weight of the animals.

Study duration: 14 days

**Preparation of dose:**

Ethanolic extract of *Leucas cephalotes* suspended in 3% CMC, to prepare a dose of 4000 mg/kg body weight of animal, and administered 1ml/100gm body weight of the animal.

**Procedure:**

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female animal (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with Ethanolic extract of *Leucas cephalotes* (4000 mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behavior or mortality. 6 hours post dosing again body weights recorded. Form the next day onwards, each day for 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

**FLOW CHART**

OECD Guidelines: Test Procedure Starting Dose of 300 mg/kg B.W

START

300 mg/ kg / 3 animals

0 - 1 (None of animals died)

2000 mg/ kg

2 – 3 (None of animals died)

3000 mg/kg (none of animals died)

4000 mg/ kg / 3 animals

None of animals died

LD50 cut off 4000 mg/ kg B.W

**Chemicals**

All the chemicals and solvents were of analytical grade. Silymarin was obtained from silybon, Micro Labs, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India. Male Albino rats weighing between 150-200 gm used in the experiment were kept in animal house under standard environmental conditions and had free access to feed and water ad libitum. The animals were fasted for 16 hours before experiment but allowed free access to water.

The rats were divided into five groups each containing four rats.

| | | |
|---|---|---|
| Group 1 | Control | Received water (5ml/kg. p.o) for 21 days once daily, and served as normal control |
| Group 2 | Negative control | Received water (5 ml/kg. p.o) for 21 days once daily and 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days. |
| Group 3 | Standard | Received 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days and standard drug silymarin (25 mg/kg. p.o.) for 21 days once daily |
| Group 4 | High dose | Received 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days and Received Ethanolic extract of *Leucas cephalotes* (400 mg/kg) 21 days once daily |

## V RESULTS

### PRELIMINARY PHYTOCHEMICAL ANALYSIS

The results of qualitative phytochemical analysis of the crude powder and the methanol extract of *Leucas cephalotes* are shown in Table.

**Table ---:** Preliminary qualitative phytochemical analysis of *Leucas cephalotes*

| Phytochemical | Test | Methanolic extract |
|---|---|---|
| Alkaloids | Dragandroffs test<br>Mayers test<br>Wagners test | +<br>+<br>+ |
| Flavonoids | Shinoda test<br>Alkaline reagent test | +<br>+ |
| Cardiac glycosides | Keller-kilianni test | - |
| Phlobotannins | HCl test | + |
| Saponins | Frothing test | + |
| Steroids | Libbermann-Burchard test | - |
| Tannins | $FeCl_3$ test | + |
| Triterpenes | $H_2SO_4$ test | + |

(-): absent, (+): present.

In methanol extract maximum amount of tannins, alkaloids, Flavonoids, phlobotannins, saponins and triterpenes were present. Cardiac glycosides and steroids were absent.

## RESULTS AND DISCUSSION

**Table 1: Effect of extracts of Ethanolic extracts *Leucas cephalotes* SGOT**

| GROUP | SGOT level mean ± SEM |
|---|---|
| Control | 1512±1.05 |
| Negative Control | 1925.01± 2.131**a |
| Standard | 1531.10±1.3**b |
| LC 200mg/kg | 1556.13±1.15 *b |
| LC 400mg/kg | 1692.21±2.05 ***b |

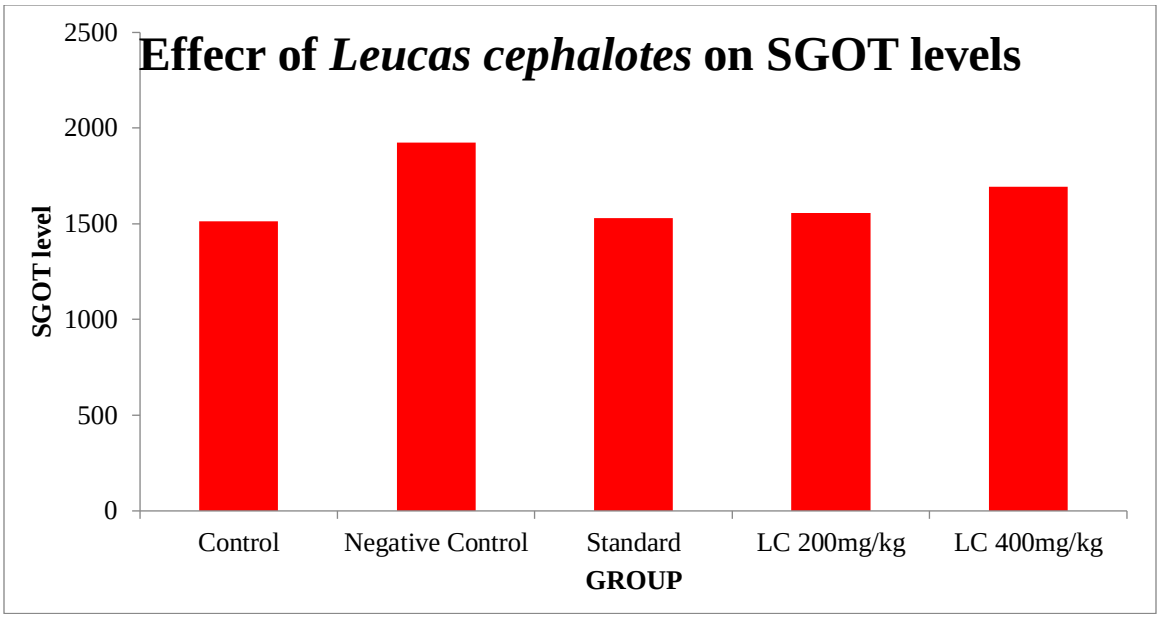


**Figure1: Graphical representation of Effect of *Leucas cephalotes* on SGOT**

Values are expressed as Mean ± SEM, n=6
Comparison: a -Group I vs. Group II b- Group II vs. Group III, IV & V; NS Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet"s "t" Test

**EFFECT OF *LEUCAS CEPHALOTES*ON SGOT**

There was significant (p<0.001) increase in serum SGOT in Ethanol induced group when compared to control group. There was significant (p<0.001) decrease in serum SGOT in Silymarin treated group when compared to control group. There was significant (p<0.001) decrease in serum SGOT in *Leucas cephalotes* treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.001) decrease in serum SGOT in *Leucas cephalotes* treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant (p<0.01) decrease in serum SGOT level in Silymarin treated rats when compared to ethanol induced. The *Leucas cephalotes* at a dose of 200mg/kg/p.o showed a significant (p<0.010) decrease in serum SGOT level when compared to ethanol induced group. The *Leucas cephalotes* at a dose of 400 mg/kg/p.o showed a significant (p<0.001) decrease in serum SGOT level when compared to Ethanol induced group.

**The results were shown in the Table no.1 and Graph no.2.**

**Table 2: Effect of extracts of Ethanolic extracts *Leucas cephalotes* on SGPT**

| **GROUP** | **SGPT level mean ± SEM** |
|---|---|
| Control | 1612±1.13 |
| Negative Control | 1814.12± 2.121**a |
| Standard | 1601.15±2.26**b |
| *Leucas cephalotes* 200mg/kg | 1589.10±1.19 *b |
| *Leucas cephalotes* 400mg/kg | 1842.20±2.51 ***b |

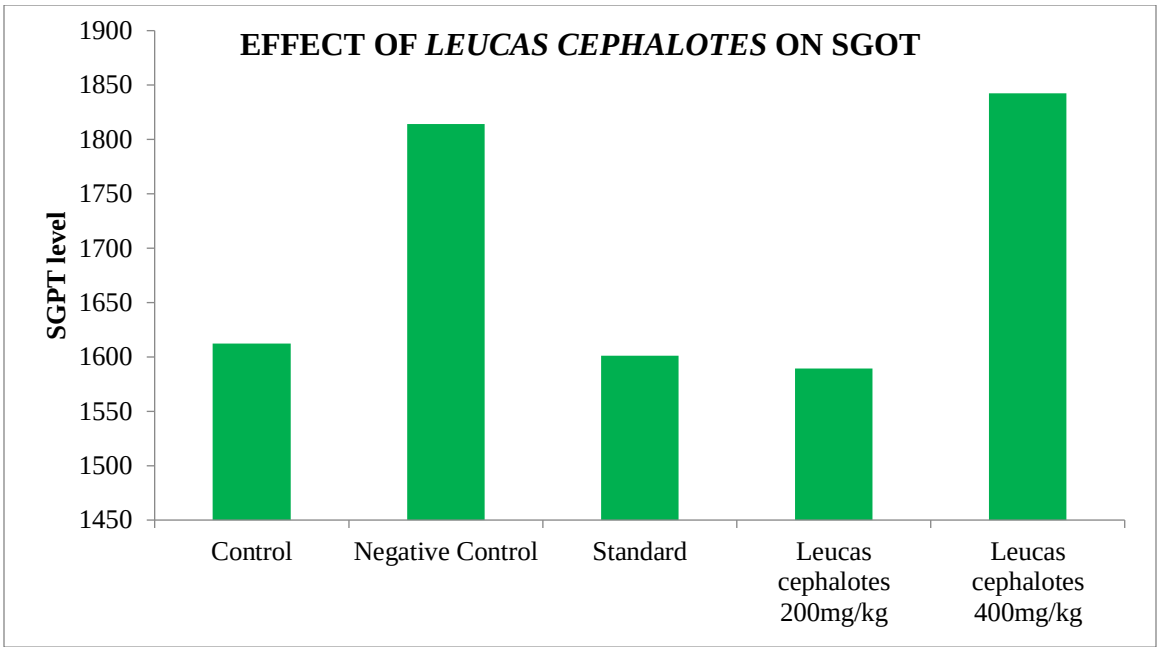


**Figure2: Graphical representation of Effect of *Leucas cephalotes* on SGPT**

Values are expressed as Mean ± SEM, n=6
Comparison: a -Group I vs. Group II b- Group II vs. Group III, IV & V; NS Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet"s "t" Test

**EFFECT of *LEUCAS CEPHALOTES* ON SGPT**

There was significant (p<0.01) increase in serum glutamic pyruvate transaminase level in Ethanol induced rats when compared to control group. There was significant (p<0.05) decrease in SGPT in ethanol treated group when compared to control group.

There was significant (p<0.01) decrease in serum SGPT in *Leucas cephalotes* treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.01) decrease in serum SGPT in *Leucas cephalotes* treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant (p<0.01) decrease in serum SGPT level in Silymarin treated rats when compared ethanol induced group. The *Leucas cephalotes* at a dose of 200mg/kg/p.o showed a significant (p<0.01) decrease in serum SGOT level when compared to Ethanol induced group. The *Leucas cephalotes* at a dose of 400 mg/kg/p.o showed a significant (p<0.001) decrease in serum SGOT level when compared to Ethanol induced group.

**Table 3: Effect of extracts of Ethanolic extracts *Leucas cephalotes* on Bilirubin**

| GROUP | Total Bilirubin mean ± SEM |
|---|---|
| Control | 1.82±0.01 |
| Negative Control | 2.96 ± 0.12***a |
| Standard | 1.56 ± 0.02*b |
| *Leucas cephalotes* 200mg/kg | 1.53 ± 0.01 **b |
| *Leucas cephalotes* 400mg/kg | 1.26 ± 0.05 **b |

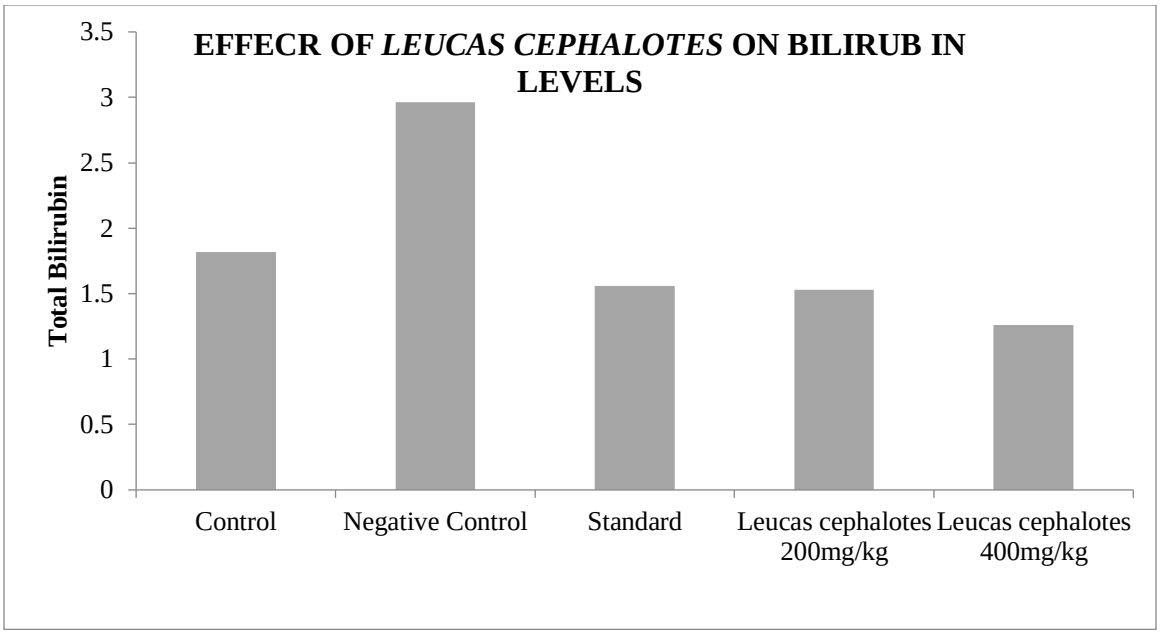


**Figure3: Graphical representation of Effect of *Leucas cephalotes* on Bilirubin**

Values are expressed as Mean ± SEM, n=6 Comparison: a -Group I vs Group II b- Group II vs Group III, IV & V; NS Non-significant;

*P<0.05, **P<0.01; ***P<0.001 One way ANOVA followed by Dunnet"s "t" Test

**EFFECT OF *LEUCAS CEPHALOTES* ON TOTAL BILIRUBIN**

There was significant (p<0.01) increase in Bilirubin level in Ethanol induced group when compared to control group. There was significant (p<0.01) decrease in Bilirubin in Silymarin treated group when compared to control group. There was significant (p<0.05) decrease in Bilirubin in *Leucas cephalotes* treated group at a dose of 200mg/kg/po when compared to control group. There was significant (p<0.001) decrease in Bilirubin in *Leucas cephalotes* treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant (p<0.01) decrease in Bilirubin in Silymarin treated rats when compared ethanol treated. The *Leucas cephalotes* at a dose of 200mg/kg/p.o showed a significant (p<0.05) decrease in serum bilirubin when compared to ethanol induced group. The *Leucas cephalotes* at a dose of 400 mg/kg/p.o showed a significant (p<0.001) decrease in Bilirubin when compared to Ethanol induced group.

**Table 4: Effect of extracts of Ethanolic extracts *Leucas cephalotes* on ALP**

| **GROUP** | **ALP level mean ± SEM** |
|---|---|
| Control | 51.02±0.03 |
| Negative Control | 54.2±0.02*a |
| Standard | 42.2±0.03**b |
| *Leucas cephalotes* 200mg/kg | 47±1.11***b |
| *Leucas cephalotes* 400mg/kg | 44.1±0.12*b |

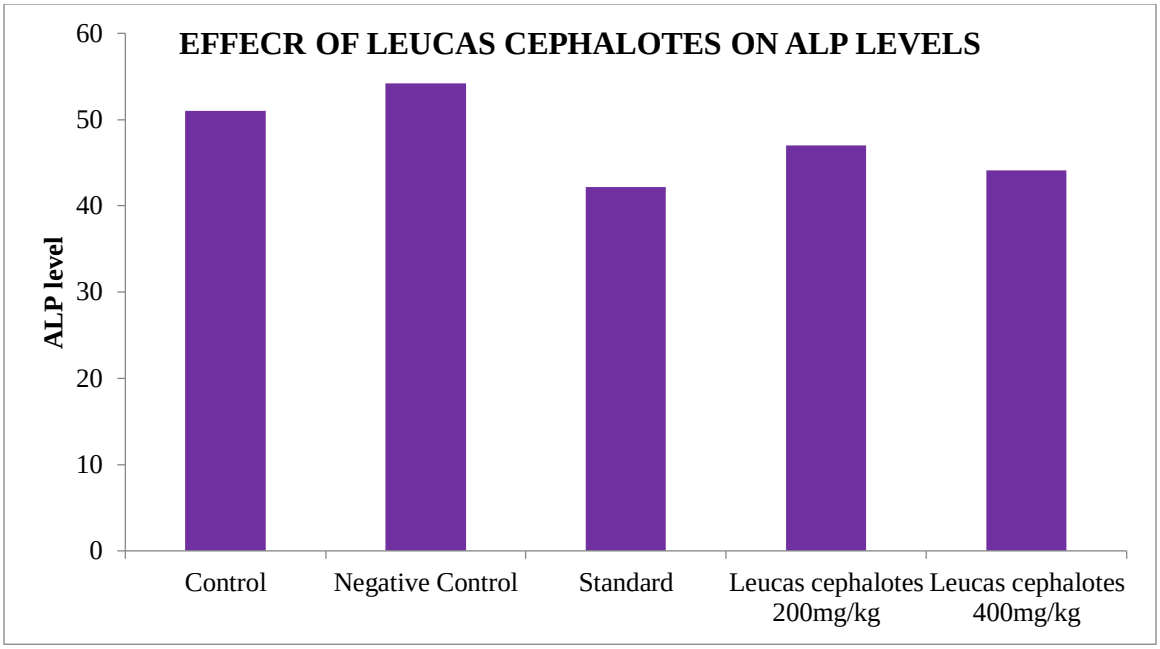


**Figure4: Graphical representation of Effect of *Leucas cephalotes* on ALP**

**EFFECT OF *LEUCAS CEPHALOTES*ON ALP**

There was significant ($p<0.01$) increase in ALP in ethanol induced group when compared to control group. There was significant ($p<0.01$) decrease in ALP in Silymarin treated group when compared to control group. There was significant ($p<0.05$) decrease in ALP in *Leucas cephalotes* treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.001$) decrease in Alp in *Leucas cephalotes* treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.05$) decrease in ALP in Silymarin treated rats when compared ethanol treated. The *Leucas cephalotes* at a dose of 200mg/kg/p.o showed a significant ($p<0.001$) decrease in ALP when compared to Ethanol induced group. The *Leucas cephalotes* at a dose of 400 mg/kg/p.o showed a significant ($p<0.05$) decrease in ALP when compared to Ethanol induced group.

**Histopathological studies of the liver in paracetamol induced hepatotoxicity**

The histopathological evaluation of paracetamol toxicity in all the groups was examined and shown in figure. The description is as follows, Section of rat liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Section of liver in toxicant control group shows partially effaced architecture. Some of the hepatocytes show apoptotic changes, perivenular mononuclear

## CONCLUSION

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study ethanol was used to induce Hepatotoxicity, since it is clinically relevant. Ethanol produces a constellation of dose related deleterious effects in the liver (Leo et al., 1982). The majority of ethanol is metabolized in the liver and individuals who abuse alcohol by routinely drinking 50-60 g (about 4 to 5 drinks) of ethanol per day are at risk for developing alcoholic liver disease. In addition, both acute and `chronic ethanol administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury. Besides the development of fatty liver (steatosis), another early sign of excessive ethanol consumption is liver enlargement and protein accumulation, both of which are common findings in alcoholics and heavy drinkers.

In order to investigate the medicinal use of *Leucas cephalotes* in hepatoprotective, we evaluated crude extract for its Hepatoprotective activity using different *in vitro* assays and *in vivo* rat model of Hepatoprotective activity.

Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic

transaminase (SGPT) are indications of hepatocellular injury. In the present study Ethanolic extract of *Leucas cephalotes* at a dose of 500 mg/kg, p.o caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by ethanol.

On the other hand suppression of elevated ALP activities with concur-rent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants. These results indicate that EEMI preserved the structural integrity of the hepatocelluar membrane and liver cell architecture damaged by ethanol which was confirmed by histopathological examination.

On examining the liver function tests of ethanol induced animals, the SGOT, SGPT, ALP, Total bilirubin has significantly increased After treatment with the ethanolic extract of *Leucas cephalotes* (200 mg/kg and 400 mg/kg) the excretion of has SGOT, SGPT, ALP, Total Bilirubin significantly decreased Although the low dose was more potent than the high dose when compared with silymarin treated group, which is a standard.

Ethanolic extract *Leucas cephalotes* of has shown promising *invitro* efficacy on Hepatoprotective activity, we have observed increase in the absorbance indicating the inhibition of Nucleation and Aggregation of calcium oxalate in *invitro* studies.

For the *in vivo* Hepatoprotective activity, of *Leucas cephalotes*, Ethanol-induced hepatotoxicity rat model of was used. Since the liver damage inducing treatment, Ethanol, was given orally, therefore, the extract was given p.o. in order to prevent any potential interaction of Ethanol with plant constituents inside gut, interfering with absorption of either of the two. Administration of Ethanol resulted in the increased toxicity, which might be due to the Hepatotoxicity, as evident by increase in SGOT, SGPT, and ALP as compared to normal.

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