EVALUATION OF *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *Erythrina indica* L., STEM BARK EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

A Dissertation submitted to

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In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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Under the guidance of

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CHENNAI – 600003

MAY 2017

CERTIFICATE

This is to certify that the dissertation entitled **"**EVALUATION OF *IN VIVO* HEPATOPROTECTIVE ACTIVITY OF *Erythrina indica* Lam., STEM BARK EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS**"** submitted by the candidate bearing the **Register No: 261526052** in partial fulfillment of the requirements for the award of degree of MASTER OF PHARMACY in PHARMACOLOGY by the Tamil Nadu Dr. M.G.R Medical University, Chennai**,** is a bonafide work done by him during the academic year 2016-2017 under the guidance of **Mrs. R. Indumathy, M.Pharm.,** Assistant professor of Pharmacology, Institute of Pharmacology, Madras Medical College, Chennai- 600 003.

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DETICATED

TO

MY FAMILY

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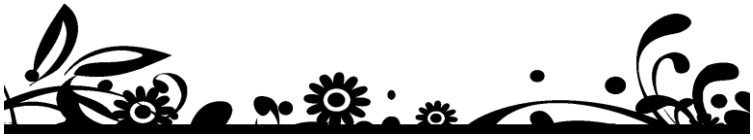
LIST OF ABBREVIATIONS

EEEI	Ethanolic extract of *Erythrina indica*
EAEEI	Ethyl acetate extract of *Erythrina indica*
PEEEI	Petroleum ether extract of *Erythrina indica*
CCl_4	Carbon tetrachloride
CCl_3	Trichloromethyl free radical
$CCl_3OO\bullet$	Trichloromethylperoxy free radical
CC_2O	Phosgene
SOD	Superoxide dismutase
LPO	Lipid peroxidase
GPx	Glutathione peroxidase
CAT	Catalase
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
TB	Total bilirubin
TP	Total protein
ALB	Albumin
INF-γ	Interferon
TNFα	Tumor necrotic factor alpha
TBA	Thiobarbituric acid

VCAM-1	Vascular cell adhesion molecule
ICAM-1	Intracellular adhesion molecule
PECAM	Platelet endothelial cell adhesion molecule
IL-8	Interleukin-8
NASH	Non-alcoholic steatohepatitis
FLD	Fatty liver disease
NAFLD	Non-alcoholic fatty liver disease
GS	Gilbert's syndrome
PSC	Primary biliary cirrhosis and Primary sclerosing cholangitis
NTD	Neglected tropical disease
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
CYP	Cytochrome P
IGF-1	Insulin-like-growth-factor
CRP	C-reactive protein
SAA	Serum amyloid A
HO-1	Heme-oxygenase-1
MIG	Monokine-induced by gamma interferon
IP-10	Gamma-interferon-inducible protein
KC	Cytokine-induced neutrophil chemoattractant
MIPs	Macrophage inflammatory proteins
HSC	Hepatic stellate cells

MCP-1	Macrophage-chemotactic-protein-1
ITAC	IFN-gamma-inducible T cell alpha chemoattractant
VAP-1	Vascular adhesion protein-1
LPS	Lipopolysaccharide
LAK	Lymphokine-activated killer cells
CBC	Complete blood count
MRI	Magnetic resonance image
CT	Computed tomography
ERCP	Endoscopic retrograde Cholangio Pancreatography
GI	Gastro intestine tract
HCV	Hepatitis C virus
HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
DPPH	2,2-diphenyl-1-picrylhydrazyl
AEEI	Aerial part of *Erythrina indica*
TC	Total cholesterol
TG	Total glyceride
TBARS	Thiobarbituric acid reactive substances



INTRODUCTION

1. INTRODUCTION

The liver plays a role of vital functions in the performance, maintenance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply and energy provision. Therefore, carrying of a healthy liver is important for the overall wellbeing of an individual.[1,2]

Liver diseases are the most serious illness and major health problem around the world. The liver toxicity mainly caused by toxic chemicals like excess consumption of alcohol, high doses of paracetamol, toxic industrial chemicals, aflatoxins, carbon tetrachloride, water pollutants, chemotherapeutic agents, peroxidised oil, etc[3]. This increases the risk of liver diseases like Hepatitis, Alcoholic liver disease, Hereditary diseases, Cirrhosis, Fatty liver disease, Non-alcoholic fatty liver disease etc. The important health complication of liver disease is rise along with the increasing in size of population. It is a major cause of mortality increasing every year and the fifth big killer in countries such as Wales and England.[4]

According to the World Health Organization (WHO) 21.96% of death occurred due to liver disease in India[3]. India has reached the 61st position in the world in liver disease. As the number of annual liver cancer and cirrhosis deaths increased by 1.25 to 1.75 million from 1990 to 2010. In 2010, hepatitis B virus was associated with 45% of liver cancer and 30% of cirrhosis deaths, whereas hepatitis C and alcohol each caused about 25% of liver cancer and cirrhosis.

Cirrhosis is well recognized and the main cause of hepatocellular carcinoma (HCC), which has an annual global incidence over half a million, and a 5 year survival of

10%, the incidence of this cancer has been steadily rising at an alarming rate, making HCC the 4th most common cancer in men and the 7th most common cancer in women in recent estimates. It is likely to contribute approximately 5.6% of all human cancers with a predicted increase in burden through 2020. Almost 85% of HCC occurs in the developing world. Hepatitis C virus related HCC is the fastest rising cause of cancer related deaths in the developed countries. It accounts for 70% to 85% of primary liver cancers. With the rising incidence of Nonalcoholic Steatohepatitis (NASH) and metabolic syndrome, these are also becoming a major concern.

Cirrhosis affects hundred to millions of patients worldwide. The overall burden of liver disease in the United States the vast majority of which is due to chronic disease with fibrosis-continues to expand, exacting a raising economic and social cost. Indeed, in the United States, cirrhosis is the most common non-neoplastic cause of death among hepatobiliary and digestive accounting approximately 30,000 deaths per year. Notably, hepatocellular carcinoma is the most rapidly increasing neoplasm in the United States and Western Europe.

Liver cancer was recognized as the 4th most common cancer in males and accounted for 37% of all infection-related cancers in females in a recent study from India. Data from 1992–2002 showed that combined liver and intrahepatic bile duct cancer ranked 12th in males and 18th in females with rates of 8.6%and 3.3% per 100,000 persons, respectively. The mortality rates for liver and intrahepatic bile duct cancers were still higher, ranking 10th for men and 13th for women.[5]

Statistics obtained from birth and death registration department of Pune Municipal Corporation, showed that an average of 35-40 people die every month of liver related

problems. Statistics revealed that nearly 2 lakh people in India die of terminal liver disease.[4,5]

In spite of the very great advances made in modern systems of medicine; no effective hepatoprotective medicine is available. Medicinal plants are great economic importance in the Indian subcontinent. The documentation of traditional knowledge especially on the medicinal use of plants in the history has provided many important drugs of the modern day. Even today, this area holds much more hidden treasure as almost 80% of human population in developing countries is dependent on plant resources for healthcare. Herbal medicines offer conventional treatments, provide safe and well-tolerated remedies for chronic illness which typically resulted from the combinations of secondary plant metabolites that are synthesized and deposited in specific parts or in all parts of the plant. Since, many synthetic drugs cause various side effect, drugs synthesized from the higher plants continue to occupy an important role in modern medicine and introduction of new therapeutic agents.[4]

1.1 Advantages of herbal medicine:

- Herbal medicines have long history of use and better patient tolerance as well as acceptance.
- Medicinal plants have a renewable source which is our only hope for sustainable supplies of cheaper medicines for the world growing population.
- Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity.
- Prolonged and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.

Scientific studies available on medicinal plants indicate that promising phytochemicals can be cure health problems. For example, the *Silybum marianum* (silymarin), *Citrus paradise*, *Vitis vinifea* L., *Opuntia ficus-indica*, *Matricaria chamomilla* have been employed for their hepatoprotective properties.[6]

Modern pharmaceuticals still contains 25% drugs derived from plants. Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardioprotectives, antibacterial, antiviral and antiprotozoal[5]. Plant drugs are known to play a vital role in the management of liver diseases. There are great in number of plants claimed to have hepatoprotective activities.[3]

Liver protective plants contain a variety of chemical constituents like coumarins, phenols, glycosides, monoterpenes, xanthenes and alkaloids. These findings provide great chances and flexibility in helping researchers identify compounds with good hepatoprotective potential.[6]

So, the development of plant based hepatoprotective drugs has been given importance in the global market. There are many plants which have not been subjected through scientific evaluation. *Erythrina indica* Lam leaves have been studied and they are known to possess hepatoprotective activity. The leaves contain rich phytoconstituents like flavonoids, isoflavonoids, coumarins, phenols. The presence of flavonoids has been reported as the reason for their hepatoprotective activity.

The stem bark not been so far subjected to hepatoprotective activity screening. The *Erythrina indica* Lam belongs to the family fabaceae. The stem bark of *Erythrina indica* Lam is also rich in flavonoids and iso-flavonoids[7]. Hence the present study was carried

out to explore the hepatoprotective efficacy of stem bark of *Erythrina indica* Lam against CCl_4 induced hepatotoxicity in rat's model.

AIM

&

OBJECTIVE

2. AIM AND OBJECTIVE

The literature review indicated that plant of *Erythrina indica* generally rich sources of antioxidants and hence may be considered to be good potential for hepatoprotective activity. From the literature review it is clear that no scientific work has so far been carried out on the stem bark of *Erythrina indica* Lam for hepatoprotective activity.

The aim and objective of the study is

- Evaluation of the phytochemical screening of various extracts of stem bark of *Erythrina indica* Lam.

- Evaluation of the hepatoprotective activity of various extracts of stem bark of *Erythrina indica* in Carbon tetrachloride induced hepatotoxicity in rats. Biochemical estimations are,
    - Estimation of Aspartate amino transferase (AST)
    - Estimation of Alanine aminotransferase (ALT)
    - Estimation of Alkaline phosphate (ALP)
    - Estimation of Total bilirubin (TB)
    - Estimation of Total protein content (TP)
    - Estimation of serum Albumin (ALB)

- Assessment of *in vivo* antioxidant property of various extracts of stem bark of *Erythrina indica* Lam.
    - Super oxide dismutase (SOD)
    - Lipid peroxidation (LP)
    - Glutathione peroxidase (GPx)
    - Catalase (CAT)

RIVIEW OF LITERATURE

3. REVIEW OF LITERATURE

3.1 DISEASE PROFILE

The liver is a dynamic organ characterized by several unique properties, including self-renewal, that permit its daily exposure to ingested nutrients, gut-derived endobiotics, and xenobiotic metabolism without adverse consequences. The unique position of the liver also confers vulnerability to wide variety of insults and injury. These are characterized by cell death, which can target any cell type in the liver. Hepatocytes, the most abundant cell type, are commonly affected in both acute and chronic liver diseases. Hepatic inflammation facilitates, accelerates, and augments liver injury[8]. Liver disease (also called hepatic disease) is a type of damage to liver or disease of the liver[9]. There are more hundred kinds of liver diseases; some of the most common ones are,

Fibrosis and cirrhosis[10,11]

Hepatic fibrosis refers to the accumulation of extracellular matrix, or scar tissue, in response to acute or chronic liver injury. The injury ultimately leads to the clinical-pathologic syndrome known as cirrhosis. From a histologic standpoint, cirrhosis can be considered the end stage consequence of fibrogenesis occurring in the hepatic parenchyma, resulting in nodules formation and blood flow and the clinical sequelae typical of cirrhosis. Both fibrosis and cirrhosis are the consequence of a sustained wound healing response to chronic liver injury from a range of causes including viral, autoimmune, drug-induced, cholestatic, and metabolic diseases. Nearly 40% of patients with histologic cirrhosis are asymptomatic and may remain so for long periods of time. However, once complications (e.g., ascites, variceal hemorrhage encephalopathy) develop, progressive deterioration leading to death or liver transplantation is typical. In

such patients there is 5-year mortality, with approximately 70% of these deaths directly attributable to liver diseases. In asymptomatic individuals, cirrhosis may be first suggested during routine examination, although histological analysis may be required to establish the diagnosis.

Hepatitis

Hepatitis refers to an inflammatory condition of the liver. It is commonly caused by a viral infection, but there are other possible causes of hepatitis. These include autoimmune hepatitis and it occurs as a secondary result of medications, drugs, toxins and alcohol. Autoimmune hepatitis is a disease that occurs when your body makes antibodies against your liver tissue.

Hepatitis A, B, and D are preventable with immunization. Medications may be used to treat chronic cases of viral hepatitis[2].There is no specific treatment for NASH; however, a healthy lifestyle, including physical activity, a healthy diet and weight loss, is important. It may be treated with medications to suppress the immune system. A liver transplant may also be an option in certain cases.

Alcoholic liver diseases

Alcoholic liver disease is a term encompasses the liver manifestations of alcohol over consumption, including fatty liver, alcoholic hepatitis and chronic hepatitis with liver fibrosis or cirrhosis.

It is the major cause of liver disease in Western countries. Although steatosis (fatty liver) will develop in any individual who consumes a large quantity of alcoholic beverages over a long period of time, this process is transient and reversible. Of all chronic heavy drinkers, only 15–20% develops hepatitis or cirrhosis, which can occur

concomitantly or in succession. The mechanism behind this is not completely understood. 80% of alcohol passes through the liver to be detoxified. Chronic consumption of alcohol results in the secretion of pro-inflammatory cytokines (TNF-alpha, Interleukin 6 and Interleukin 8 [IL8]), oxidative stress, lipid peroxidation, acetaldehyde toxicity and decreasing antioxidant enzymes levels. These factors cause inflammation, apoptosis and eventually fibrosis of liver cells.

Fatty liver disease

Fatty liver is a reversible condition where in large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (i.e., abnormal retention of lipids within a cell). Despite having multiple causes, fatty liver can be considered a single disease that occurs worldwide in those with excessive alcohol intake and obese (with or without effects of insulin resistance). The condition is also associated with other diseases that influence fat metabolism[2]. When this process of fat metabolism is disrupted, the fat can accumulate in the liver in excessive amounts, thus resulting in a fatty liver. It is difficult to distinguish alcoholic FLD from nonalcoholic FLD, and both show microvesicular and macrovesicular fatty changes at different stages.

Accumulation of fat may also be accompanied by a progressive inflammation of the liver (hepatitis), called steatohepatitis. By considering the contribution by alcohol, fatty liver may be termed alcoholic steatosis or nonalcoholic fatty liver disease(NAFLD), and the more severe forms as alcoholic steatohepatitis (part of alcoholic liver disease) and non-alcoholic steatohepatitis (NASH).

Nonalcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is one of the types of fatty liver which occurs when fat is deposited (steatosis) in the liver due to causes other than excessive alcohol use. NAFLD is the most common liver disorder in developed countries. NAFLD is related to insulin resistance and the metabolic syndrome and may respond to treatments originally developed for other insulin-resistant states (e.g. diabetes mellitus type 2) such as weight loss, metformin, and thiazolidinediones. Up to 80% of obese people have the disease. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD, and is regarded as a major cause of cirrhosis of the liver of unknown cause.

Hereditary disease

Hereditary diseases that cause damage to the liver include hemochromatosis, involving accumulation of iron in the body and Wilson's disease. Liver damage is also a clinical feature of alpha 1- antitrypsin deficiency and glycogen storage disease type II.

Gilbert's syndrome

Gilbert's syndrome (GS) is a common genetic liver disorder. It produces elevated levels of unconjugated bilirubin in the bloodstream (hyperbilirubinemia), but this normally has no serious consequences, although mild jaundice may appear under conditions of exertion or stress.

The cause of this hyperbilirubinemia is the reduced activity of the enzyme glucuronyltransferase, which conjugates bilirubin and a few other lipophilic molecules. Conjugation renders the bilirubin water-soluble, after which it is excreted in bile into the

duodenum. There are a number of variants of the gene for the enzyme, so the genetic basis of the condition is complex.

Autoimmune disorder

Sometimes the immune system may begin to attack the liver or bile ducts causing inflammation and scaring which leads to a progressive form of liver disease. Examples of liver disease believed to be caused by the immune system are primary biliary cirrhosis (PSC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis.

Fascioliasis

Fasciolosis (also known as fascioliasis, distomatosis and liver rot) is a parasitic worm infection caused by the common liver fluke *Fasciola hepatica* as well as by *Fasciola gigantica*. The disease is a plant-borne trematode zoonosis and is classified as a neglected tropical disease (NTD). It affects humans, but its main host is ruminants such as cattle and sheep. The disease progresses through four distinct phases; an initial incubation phase of between a few days up to three months with little or no symptoms; an invasive or acute phase which may manifest with: fever, malaise, abdominal pain, gastrointestinal symptoms, urticaria, anemia, jaundice and respiratory symptoms. The disease later progresses to a latent phase with less symptoms and ultimately into a chronic or obstructive phase months to years later.[1,5] In the chronic state the disease causes inflammation of the bile ducts, gall bladder and may cause gall stones as well as fibrosis. While chronic inflammation is connected to increased cancer rates; it is unclear whether Fascioliasis is associated with increased cancer risk.

Causes of liver damage

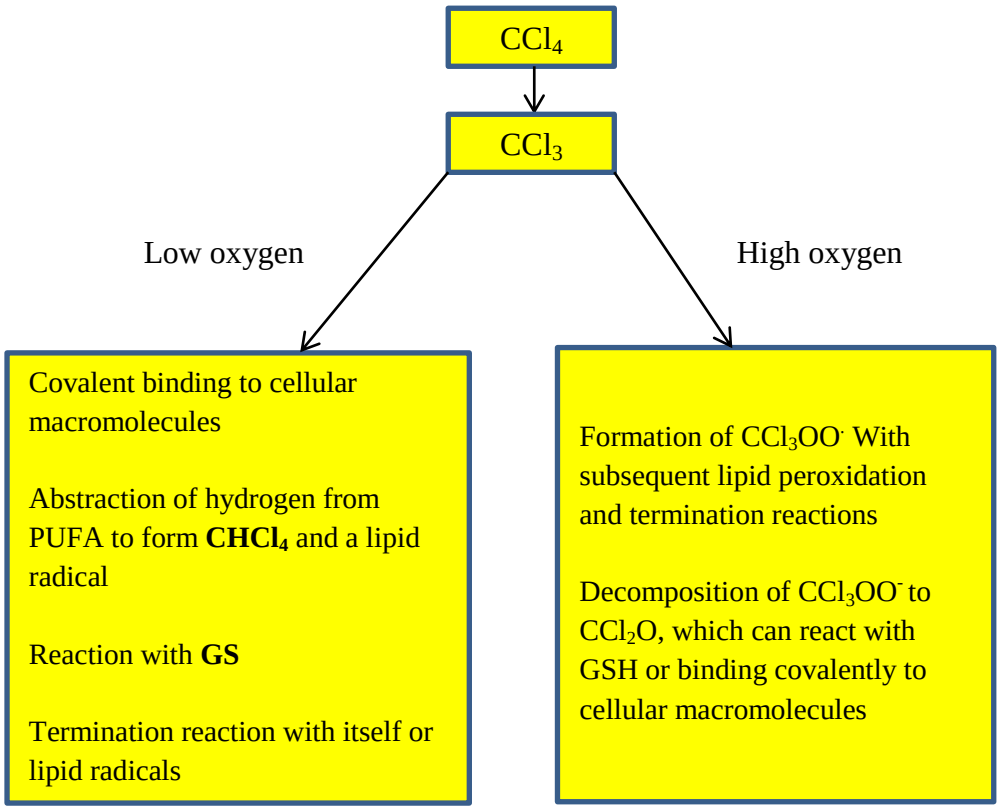
Chemical induced liver damage: carbon tetrachloride, alcohol consumption, aflatoxins, 1, 1, 2, 2-tetrachloroethane, carbon tetrabromide, dimethyl formamide, ethylene dichloride.

Mechanism of Chemical induced hepatotoxicity[10]

Most of the chemicals produce hepatotoxicity by oxidative stress mechanism. Oxidative stress is an imbalance between oxidants and anti-oxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage. A key mechanism involves reactive oxygen species (ROS), reactive nitrogen species (RNS), and a range of free radicals generated by bioactivation of xenobiotics. All of the classes of cellular macromolecules can be the target of oxidant-induced liver injury. Proteins are most often considered the critical targets in acute necrosis, but oxidants also genobiotic. Non radical oxidants, such as hydrogen peroxide and lipid hydroperoxides, are quantitatively most important under most conditions; however, free radicals can also be important in mediating hepatotoxicity.

Free radicals can be generated in the liver in several ways. CYP enzyme generate radicals from xenobiotics by different mechanisms: 1-electron oxidation to form a cation radical ($R \longrightarrow \bullet\mathbf{R}^{+} + e^{-}$); 1-electon oxidation to form a cation radical ($R + e^{-} \longrightarrow \bullet\mathbf{R}^{-}$); or hemolytic bond scission to yield a neutral radical ($R^{-} \longrightarrow \bullet\mathbf{R} + \bullet\mathbf{R}$). Hepatotoxicants of occupational/environmental (e.g.; CCl_4) as well as of clinical importance (e.g. halothane) are bioactivated in liver to free radical species. CCl_4 toxicity provides a useful model and is representative of a large number of halogenated hydrocarbons that can be similarly activated.

Figure.1: Hepatic metabolism and oxygen-dependent effects of carbon tetrachloride (CCl_4)



CCl_4 is a prototypical hepatotoxin that causes centrilobular necrosis and associated fatty liver. Caspase-3 is activated and release in to the plasma with a time course suggesting initial activation of apoptosis followed by secondary necrosis. A primary event in the pathogenesis is the reductive dehalogenation of CCl_4 to the trichloromethyl free radical ($\bullet CCl_3$) by hepatic mixed function oxidases (**fig.1**). The free radical $\bullet CCl_3$ can initiate lipid peroxidation, and in the presence of oxygen it forms the more reactive trichloromethylperoxy free radical ($CCl_3OO\bullet$), which also decomposes to phosgene (CC_2O). The lipid peroxidation in the liver associated with CCl_4 has been viewed as a critical event because it occurs early and is associated with reductions of enzyme activities and inactivation of the Ca^{+} concentration establishes conditions for activation of the mitochondrial permeability transition, with associated cytochrome c release and caspase activation. In addition to free radicals of the parent

compound, other reactive oxygen and reactive nitrogen species are often involved in hepatotoxicity.

Alcohol abuse

Drug induced: more than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Drug induced liver injury is responsible for 5% of hospitalized persons and 50% of all acute liver failures. Some of them are Acetaminophen over dose, Amiodarone, Ketoconazole, Rifampicin.

Virus induced: Hepatitis A, B, C, D and E.

Other causes; Non-alcoholic fatty liver, malnutrition, extra hepatic infections, ingestion of poisonous wild mushrooms and haemochromatosis.

Pathophysiology of liver damage[12]

Hepatocytes make up 70-80% of the cytoplasmic mass of the liver. These cells are involved in protein synthesis, protein storage and transformation of carbohydrates, synthesis of cholesterol, bile salts and phospholipids, and detoxification, modification and excretion of exogenous and endogenous substances. The hepatocyte also initiates the formation and secretion of bile. Hepatocytes are able to synthesize hormones, like insulin-like-growth-factor IGF-1, thrombopoietin, and also erythropoietin. They also synthesize cytokines like interleukin (IL)-8 and respond to acute phase mediators like IL-6, with the synthesis of acute phase proteins like C-reactive protein (CRP) or serum amyloid A (SAA) and many others. The cells possess different intracellular defense proteins like heme-oxygenase-1 (HO-1).

When however the defense mechanisms are not sufficient to withstand the damaging attacks cells start to synthesize chemokines (CXC-chemokines like: monokine-induced by gamma interferon (MIG), gamma-interferon-inducible protein (IP-10), cytokine-induced neutrophil chemoattractant (KC) and macrophage inflammatory proteins (MIPs) (MIP-1, MIP-2, MIP-3), which are supposed to be responsible for attraction of inflammatory cells like granulocytes and mononuclear phagocytes and to activation of resident macrophages (Table-1, Fig. 1). In the attempt to eliminate the damaging toxin the defense response however leads to death of the stressed hepatocyte.

Hepatic stellate cells (HSC) and liver (myofibroblasts) have modulatory roles in inflammatory conditions, based on their capability of cytokine and chemokine production. The quiescent Stellate (Ito) cells (HSC) store vitamin A, but produce extracellular matrix and collagen when activated. They are located in the space of Disse between hepatocytes and endothelial cells.

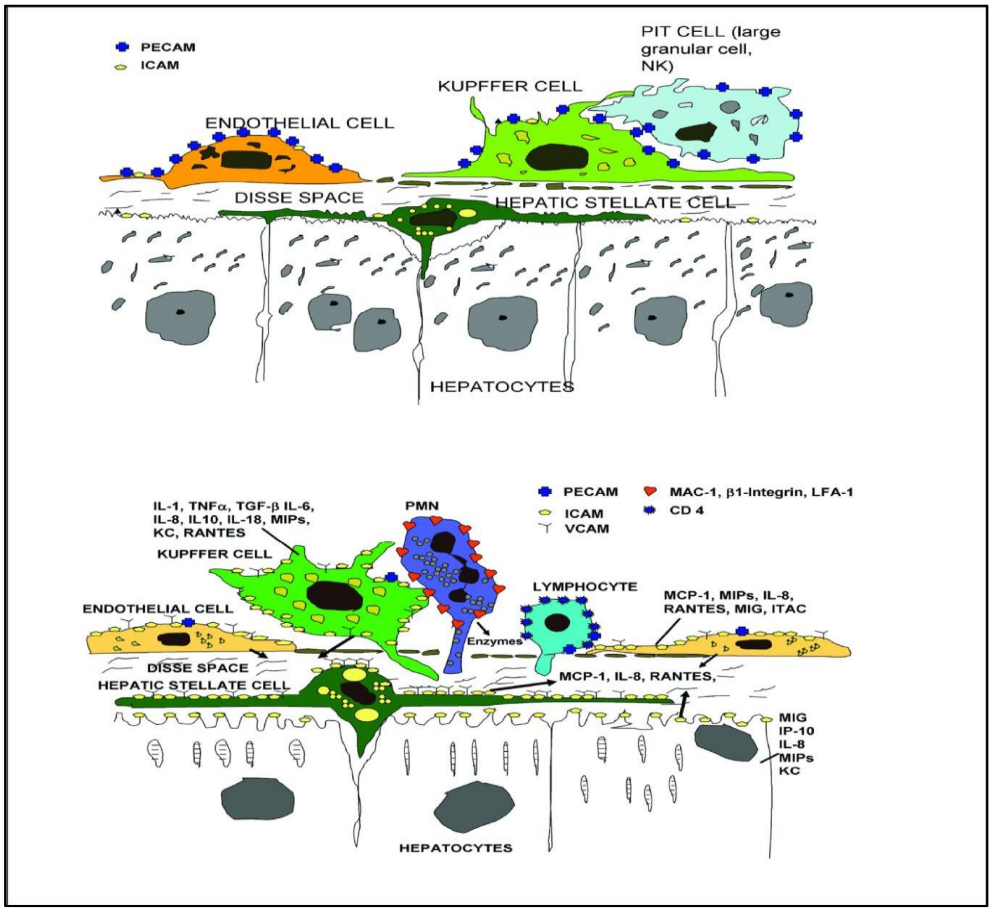
Hepatic stellate cells might also play a role during liver inflammation. ICAM-1 and VCAM-1 expression was present in HSC *in vitro* and in cells located in the sinusoidal/perisinusoidal area of normal liver. Growth factors, e.g., transforming growth factor-β1, down-regulated ICAM-1- and VCAM-1-coding mRNAs and stimulated N-CAM expression of HSC. In contrast, inflammatory cytokines like tumor necrosis factor-alpha reduced N-CAM-coding mRNAs, whereas induced of ICAM-1- and VCAM-1-specific transcripts by several fold. HSC might be important during the onset of hepatic tissue injury by modulating the recruitment and migration of mononuclear cells within the perisinusoidal space of diseased livers.

In addition, the secretion of several cytokines and chemokines was demonstrated in hepatic stellate cells including MCP-1, RANTES, IL-8 (Table 1, Fig.1). Sinusoids display a discontinuous, fenestrated endothelial cell lining. The sinusoidal "wall" does not possess a basement membrane and the endothelial cells are separated from the hepatocytes by the space of Disse which drains lymph into the portal tract lymphatics. Under normal conditions the hepatic sinusoidal endothelial cells express low levels of Rantes, macrophage-chemotactic-protein-1 (MCP-1), IL-8 and MIP-1α. These factors are involved in the routine leukocyte recirculation and immunological surveillance. During inflammation the chemokine expression profile of the normal hepatic endothelium changes. These changes are characterized by expression of high levels of MIP-1β, IP-10, MIG and IFN-gamma-inducible T cell alpha chemoattractant (ITAC) (Table 1, Fig. 2). Similarly to the chemokine profile the expression pattern of adhesion molecules also changes in the endothelial cells. It also expresses platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular adhesion protein-1 (VAP-1) and intercellular cell adhesion molecule-2 (ICAM-2) under normal conditions of hepatic sinusoidal endothelial cells. During inflammation this expression pattern changes, characterized by the downregulation of PECAM-1, and upregulation of ICAM-1, of vascular cell adhesion molecule VCAM-1, and of P and E selectins.

Table – 1: Induction of chemical mediators in liver cells populations during liver inflammation

Liver cells	Mediators
Hepatocytes	IL-8, IP-10, MIG, MIP-1, MIP-2, MIP-3, KC
Sinusoidal Endothelial Cells	RANTES, MCP-1, IL-8, MIP-1α, MIP-1β; MIG, ITAC
Kupffer Cells	IL-1, IL-6, IL-10, IL-18, TNF-α, TGF-β, MIPs, IL-8, IP-109, KC/GRO, RANTES
Hepatic Stellate Cells	IL8, RANTES, MCP-1

Fig.2: Sinusoidal structure in normal liver (A) and in liver inflammation (B)



The red blood cell is broken down by phagocytic action and the hemoglobin molecule is split. The globin chains are reutilized while the iron containing portion or heme is further broken down into iron which is reutilized and bilirubin, which is conjugated with glucuronic acid within hepatocytes and secreted into the bile.

During liver injury induced by hepatotoxins or by Gram-negative bacterial lipopolysaccharide (LPS), or in association with sensitizers such as D-galactosamine, CCl_4, dimethylnitrosamine, acetaminophen, alcohol, etc. the Kupffer cells get activated. Activation of Kupffer cells results in secretion of a large number of chemical mediators (cytokines: IL-1, IL-6, IL-8, TNF-α, etc. chemokines: C-X-C chemokines: MIP-2, IP-109, KC/GRO; C-C chemokines: MIP-1α, MCP-1, RANTES), most of which can induce liver injury either by acting directly on the liver cells or via chemoattraction of extrahepatic cells (e.g. neutrophils and lymphocytes) (Table 1, Fig. 2). During inflammatory conditions the expression pattern of adhesion molecules is also changed in the Kupffer cells, similarly to the sinusoidal endothelial cells. The most characteristic change is the downregulation of PECAM-1 and the upregulation of ICAM-1 (Fig. 2).

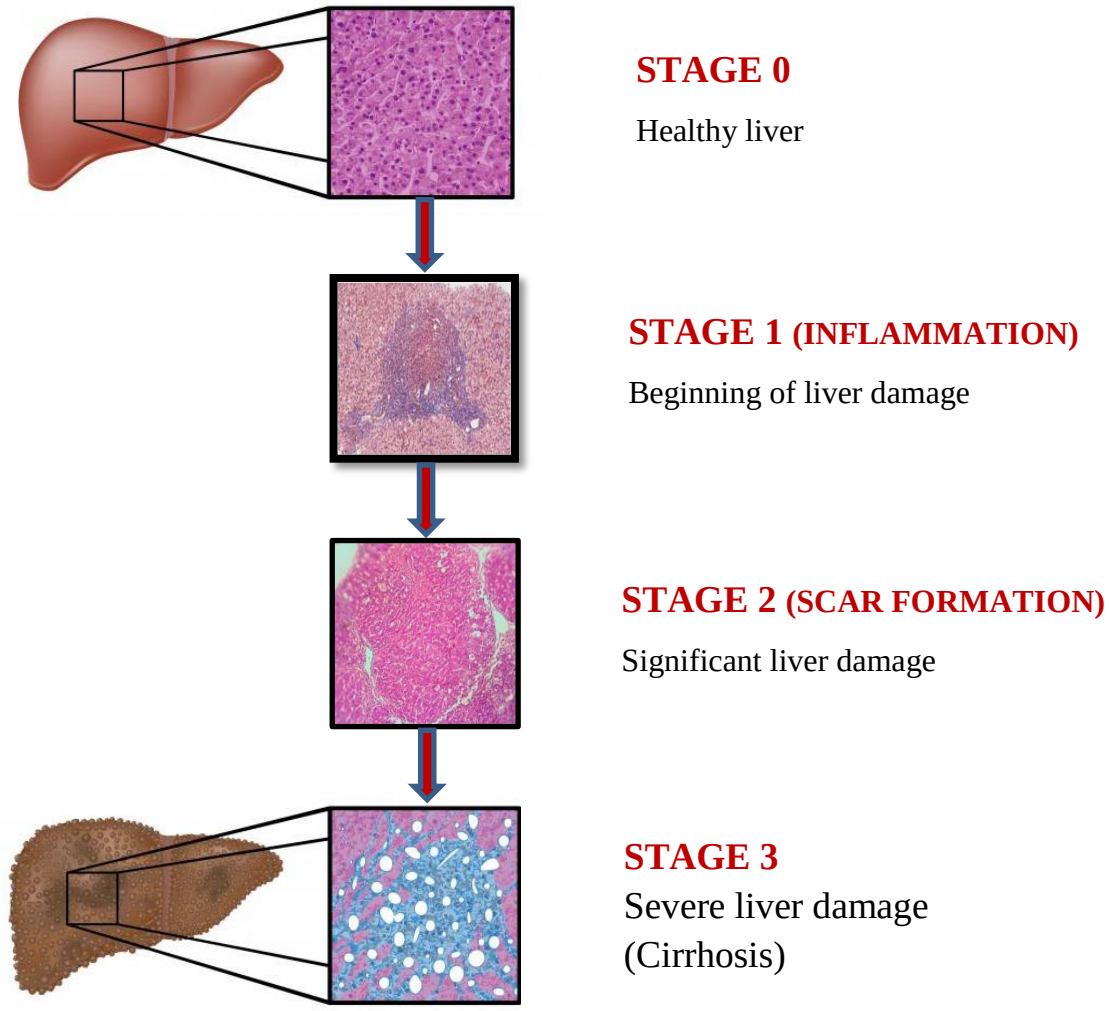
Besides synthesis of IFN-γ upon triggering by the damaging noxae, the most important function of the NK cells is the destruction of virus-infected and malignant cells without previous activation. NK cells are able to migrate and transmigrate through epithels. NK cells can be activated by interleukin-2. The resulting cell population is known as lymphokine-activated killer cells (LAK).

The chemical mediators released by Kupffer cells and by hepatocytes attract extrahepatic cells to the liver. Neutrophils (PMN) are the characteristic cellular compound of the chemoattracted cells, and are involved in the acute inflammation (Fig.2). They are always present in the inflammatory infiltrate of chronic liver disease. However, neutrophil infiltration is most prominent in alcoholic hepatitis and extravasation and transmigration of neutrophils into the liver tissue are critical for neutrophil-induced injury and cytotoxicity.

Up to now the role of T-lymphocytes in liver disease is still not clear. Previously a role was suggested of T-cells in liver injury by activating Kupffer cells to produce TNF-α However there is also a considerable amount of data demonstrating that T-cell activation against liver antigens (after a transient cellular immune attack) induces tolerance and not immunity and a recent study suggests that at least natural killer T-cells might not concern in immune-mediated liver injury. Furthermore, other studies described the liver as graveyard for T-cells.

Stages of liver damage;

Fig. 3



Signs and symptoms of the live disease [13,14]

Symptoms partly depend on the type and the extent of liver disease. In many cases, there may be no symptoms. Signs and symptoms that are common to a number of different types of liver disease include Jaundice or yellowing of the skin, Darkened urine, Nausea, vomiting, Loss of appetite, Unusual weight loss or weight gain, Diarrhoea, Light-coloured stools,Abdominal pain in the upper right part of the stomach, Generalized itching, Varicose veins (enlarged blood vessels), Hypoglycaemia (low blood sugar), Muscle aches and pains and depression

Risk factors

A numbers of factors increase the risk of developing liver disease, which can lead to liver failure. Risk factors include alcoholism, coronary artery disease (due to atherosclerosis or hardening of the arteries, or other causes), diabetes, Exposure to certain toxins, such as arsenic, Exposure to hepatitis, High triglyceride levels in the blood, Long-term treatment with corticosteroids and Obesity.

Some of the drugs currently available in the management of liver disease are,

Silymarin[15]

Silymarin is drived from the milk thistle *silybum marianum.* The milk thistle extracts has been shown to reduce lipid peroxidation and inhibit fibrogenesis. The compound has been found to be safe, but reportedly has fixed effects. In one study examining Silymarin use in alcoholics, mortality was reduced; patient with early stages of cirrhosis appeared to benefit. However, in another study in alcoholics, no survival benefit could be identified. Silymarin appeared to be safe and is commonly used by patients with fibrosing liver diseases.

Mechanism of action

Silymarin hepatoprotective effects are purportedly accomplished via several mechanisms; these include:

- Anti-oxidation
- Inhibition of lipid peroxidation
- Stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration
- Enhanced liver detoxification via inhibition of phase I detoxification
- Enhanced glucuronidation and protection from glutathione depletion
- Anti-inflammatory effects, including inhibition of leukotriene and prostaglandin synthesis, kupffer cell inhibition, mast cell stabilization, and inhibition of neutrophil migration
- Slowing or even reversing of fibrosis by reduction of the conversion of hepatic stellate cells into myofibroblasts
- Anticarcinogenesis by inhibition of cyclin-dependent kinases and arrest of cancer cell growth
- Silymarin is also found to have immunomodulatory effects on the diseased liver

Clinical application

Silymarin has been used in the management of chronic hepatic diseases in humans such as primary biliary cirrhosis, biliary disease secondary to cystic fibrosis, non-alcoholic steatohepatitis, idiopathic chronic hepatitis, autoimmune hepatitis, primary sclerosing cholangitis, and alcoholic hepatitis.

Polyenylphosphatidylcholine

Polyenephosphatidylcholine is a mixture of polyunsaturated phosphatidylcholine, extracts from soyabeans. This compound has antioxidant properties and oxidant stress is believed to be important in the inflammatory and fibrogenic response to injury, particularly in alcoholic liver disease. Because oxidative stress leads to lipid peroxidation and lipid peroxidation in injurious at the level of the cell membrane phosphatidylcholine has been proposed to be protective of injury to cell membranes, resulting in reduced cellular injury and fibrogenesis.

ANTIOXIDANT AGENTS[10]

Oxidative stress is thought to play an important role in injury, stellate cell activation, and stimulation of extacellular matrix production as described previously. Thus a wide variety of antioxidants have received attention as potential antifibrotics.

Vitamins E

Vitamins E are a lipid-soluble antioxidant that presumably protects cell membranes from oxidative stress, although the precise mechanism by which is unknown.

Other antioxidant

Malotilate is another potential cytoprotective agent, perhaps acting via inhibition of cytochrome P-450 2E1; on addition, this compound may have anti-inflammatory properties. Although malotilate was found to diminish plasma cell and lymphocytic infiltrate and piecemeal necrosis in patients with primary biliary cirrhosis, it had no significant effect on fibrogenesis.

Another agents used to antagonize oxidative stress is **S-adenosylmethionine**; this compound is important in the synthesis of the antioxidant glutathione. The enzyme responsible for its synthesis (methionine adenosyltransferase) is reduced in the injury; thus it has been hypothesized that if S-adenosylmethionine was replaced, then injury and fibrogenisis might be slowed.

Propylthiouracil is an antithyroid drug that reacts with some of the oxidized species derived from the respiratory burst and thus may be protective in alcoholic liver disease, a disease in which an increase in hepatic oxygen consumption may predispose the liver to ischemic injury.

CYTOPROTECTIVE AGENTS

Ursodeoxycholic Acid

Ursodeoxycholic acid binds to hepatocyte membranes, where it presumably stabilizes them and is thus cytoprotective. Such cytoprotective action theoretically reduces inflammation and may in turn have a beneficial effect on fibrogenesis. Although the compound has been examined extensively up to date neither experimental data nor human studies indicate that ursodeoxycholic acid has a primary antifibrotic effect in the liver.

Mechanism of action

It is more hydrophilic and hepatoprotective than the major circulating bile acids in humans. The immunomodulatory effects of ursodeoxycholic acid are to involve decreased immunoglobulin production by B lymphocytes, decreased interleukin-1 and interleukin-2 production by T lymphocytes, decreased expression of hepatocyte cell

surface membrane HLA Class 1 molecules and possibly stimulation of the hepatocyte glucocorticoid receptor.

Clinical applications

Ursodeoxycholic acid has been used in the management of chronic hepatic diseases in humans such as primary biliary cirrhosis, biliary disease secondary to cystic fibrosis, non-alcoholic steatohepatitis, idiopathic chronic hepatitis, autoimmune hepatitis, primary sclerosing cholangitis, and alcoholic hepatitis.

Adverse effects

Diarrhoea, Vomiting

Penicillamine

Penicillamine is a degradation product of penicillin but has no antimicrobial activity. It was first isolated in 1953 from the urine of a patient with liver disease who was receiving penicillin.

Penicillamine is a monothiol chelating agent which is used in veterinary medicine in the treatment of copper-storage hepatopathy (e.g. Bedlington Terriers), lead toxicity and cysteine urolithiasis. It has also been used in the management of rheumatoid arthritis and Wilson;s disease.

Dosage and formulations

For management of copper-associated hepatopathy, a dose of 10-15 mg/kg q12h p.o is given.

Adverse effects

- GIT adverse effects are common resulting in nausea and vomiting. Smaller doses on a more frequent basis may alleviate adverse effects. Aldernatively, the drug can be given with food although this will reduce absorption.

Other adverse effects observed infrequently or rarely includes,

- Fever
- Lymphadenopathy
- Skin hypersensitive reaction
- Immune-complex glomerulonephopathy

Anti-inflammatory compounds[6]

Many liver diseases such as HCV have an important inflammatory component. Inflammation in these disorders typically drives stellate cell activation and fibrogenesis, and these diseases in particular have been studied in order to evaluate the efficacy of anti-inflammatory drugs.

Cordicosteroids

Classic examples of the benefits of steroids include the improvements seen in patients with autoimmune hepatitis and alcoholic hepatitis. It could be argued that these two diseases are driven largely by inflammation, and thus the anti-inflammatory action of steroids serves to treat the underlying process. Not with standing, in patients with autoimmune hepatitis who respond to medical treatment (prednisone or equivalent), advanced fibrosis and cirrhosis are reversible. Fibrosis may improve in patient with alcoholic liver disease who respond to cordicosteroids appear to have antifibrotic effects in patients with certain liver disorders.

Interleukin-10

Interleukin-10 (IL-10) has both anti-inflammatory and immunosuppressive effects. IL-10 has been shows to reduce production of proinflammatory cytokines, such as TNF-α, IL-1, INF-ɣ, and IL-2 from T cell. These cytokines belong to the TH1 family. Endogenous IL-10 reduces the intrahepatic inflammatory response, shifts the e cytokine milieu towards TH2 predominance, and reduces fibrosis in several *in vivo* models of liver injury.

TNF inhibitors

Because TNF-α amplifies inflammation in many diseases, and because TNF-α is up-regulated in liver diseases such as alcoholic hepatitis, an anti- TNF-α compound should theoretically reduce inflammation and the stimulus for fibrosis.

The current available drugs, though useful, are also associated with side effects.

3.2 IMPORTANT OF HERBS IN TREATING LIVER DISORDER

Herbal medicines are in great demand in the developed world due to their efficacy, safety and lesser side effects. Herbal drugs are most widely used than allopathic drugs as hepatoprotective because they are inexpensive, have better curable acceptability, better compatibility with the human body and minimal side effects.

A number of plants and traditional formulations are available for the treatment of liver diseases. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity[16].It is estimated that about six hundred (600) commercial herbal formulations are used over as hepatoprotective drugs.

Amongst the many plants that have hepatoprotective activity, silymarin is widely used as a hepatoprotectant around the world. It is a mixture of flavonoid complexes and is the active component of milk thistle plant that protects liver and kidney cells from toxic effects of drugs, including chemotherapy[17]. Silymarin is composed of a mixture of four isometric flavonolignans: silibinin, isosilibinin, silydianin and silychristin. Silymarin possesses free radical scavenging ability, it increases antioxidant enzymes such as superoxide dismutase (SOD) and catalase and inhibit lipid peroxidation. It is reported to offer protection against various chemical hepatotoxins such as CCl_4 and alcoholic liver.[18]

There are many plants which have not been subjected to scientific evaluation. One such plant is *Erythrina indica* Lam it's belongs to the family of fabaceae. In this plant the leaves have been studied and they are known to possess hepatoprotective activity. The leaves contain rich phytoconstituents like flavonoids, isoflavonoids, flavones, coumarins, phenols[19,20,21]. The presence of flavonoids has been reported as the reason for their hepatoprotective activity.

The stem bark not been so far subjected to hepatoprotective activity screening. Hence the present study was carried out to explore the hepatoprotective efficacy of stem bark of *Erythrina indica* Lam against CCl_4 induced hepatotoxicity in rat's model.

3.5 PHARMACOLOGICAL INVESTIGATION

Mujahid M *et al*., conducted a research study to determine hepatoprotective potential of leaves of *Erythrina indica* (methanolic extract) against anti-tubercular drugs induced hepatotoxicity in experimental rats. Hepatoprotective potential assessed by evaluating histological examination, biochemical parameters SGOT, SGPT, ALP,

bilirubin, total protein, albumin, LDH. *In vivo* antioxidant activities such as SOD, CAT, GSH and LPO were studied. Histopathology of liver tissue showed that *Erythrina indica* attenuated the hepatocellular necrosis, regeneration and repair of cells toward normal. From the result, it was showed the extract had strongly indicated the protective effect of *Erythrina indica* against liver injury which may be attributed to its hepatoprotective activity.[22]

A study was conducted by Yashwant Kumar A *et al*., with different extracts of *Erythrina indica* stem bark to evaluate Hypoglycaemic and anti-diabetic activity in normal and alloxan-induced diabetic rats. Effect was assessed by parameters like mortality, FBG, insulin levels, blood glucose level, body weight and Oral glucose tolerance test. The data showed that the aqueous extracts treatment caused a reduction in BGL when compared to the alcoholic extract, indicating the potency of aqueous extract. These results indicated that *E. indica* possesses hypoglycaemic activity on repeated administration in alloxan induced diabetic rats.[23]

Kamalraj R *et al*., conducted a research study to determine the effect of different extracts of *Erythrina indica* on stress induced alteration on lipid profile in rats. Effect was assessed by parameters like serum lipid levels (TC) and HDL cholesterol, LDL & VLDL cholesterol. In control group stress increased the lipid levels in rats significantly except HDL cholesterol. When *Erythrina indica* treated rat's serum lipid levels increased significantly except HDL cholesterol. This study indicates that various fraction of the ethanolic extract of *Erythrina indica* is effective in attenuating stress induced dyslipidemia in rats.[24]

A study was conducted by Ajay Kumar P *et al*., with two different extracts of *Erythrina indica* bark to evaluate *In-vitro* antioxidant and anti-inflammatory activities. Standard Methanolic and ethyl acetate extracts of *Erythrina indica* was tested in vitro for its anti-inflammatory activity and antioxidant activity using anti denaturation and reducing power methods respectively. The Methanolic extract possesses significant anti-inflammatory and antioxidant effect where as ethyl acetate extract possesses moderate anti-inflammatory and antioxidant activity.[25]

A study was conducted by mohd mujahid *et al*., with methanolic extract of *Erythrina indica* to evaluate *in vitro* free radical scavenging activity by using 1,1diphenyl, 2 picryl hydrazyl assay (DPPH), reducing power and nitric oxide scavenging activity. This study indicates that *E. indica* is a potential source of natural antioxidant.[26]

Meena Agarwal *et al*., conducted a research study to determine antimicrobial potential *Erythrina indica*. The various plant part (root, stem, and leaf) of extracts of *E. indica* were screened for antibacterial activity and antifungal activity against selected test microorganisms. Result indicated that all extracts showed significant antibacterial activity against *P. funiculosa, F. moniliformae*. Among all extracts only stem part of *E. indica* showed antifungal activity against *P. funiculosum*. From the result, it was reveals that extracts of *E. indica* are showing antimicrobial potential against tested microorganism.[27]

Suthar S *et al*. evaluated to determine analgesic activity of aqueous extract of aerial part of *Erythrina indica* (AEEI) in Rodents. AEEI was evaluated for both peripheral and central analgesic activities by acetic acid induced writhing, tail immersion

and hot-plate methods. The evaluating parameters are Writhing reflex, Jumping hind paw licking response and withdrawn of tail from hot water. From the results AEEI exhibited significant analgesic activities on experimental animals as compared to the standard.[28]

A study was conducted by Jesupillai M *et al*., to evaluate the anticonvulsant effects of different extracts of leaves of *Erythrina indica* Lam on electrically and chemically induced seizures. From the result all the extracts possessed significant ($P<0.05$) anticonvulsant activity. Ethanol extract has been found to have prominent activity when compared to other extracts.[29]

Verma S M *et al*., conducted the study to evaluate the anti-inflammatory and sedative, hypnotic activity of the leaves of *Erythrina indica* Lam in albino rats in which the plants were subjected to successive extraction with methanol. Carragenan induced paw oedema model used for study of anti-inflammatory effect and Pentobarbital sodium induced sleep method. The extract exhibited significant anti-inflammatory and mild sedative activity.[30]

Vadivel *et al*.**,** studied the antioxidant potential and Type II diabetes related functionality using methanolic extract of *Erythrina indica* Lam., plant seeds. Ferric reducing/antioxidant power assay, β-carotene degradation assay, DPPH assay, superoxide radical scavenging assay, starch digestion bioassay and inhibition of α-amylase and α- glucosidase enzyme activities were performed. From the result methanolic extract showed marked antioxidant potential and Type II Diabetes related functions.[31]

A study was conducted by Soniya S *et al*., with ethanolic extract of *Erythrina indica* leaves to evaluate antihyperlipidemic activity in high fat diet which induced

hyperlipidemia in rats. The effect was assessed by parameters like TC, TG, LDL, VLDL and HDL levels. Ethanolic extract of *Erythrina indica* leaf at two dose levels i.e., 200mg/kg and 400mg/kg for 28 days resulted in the reduction of TC, TG, LDL, VLDL and HDL level in the high fat diet which induced hyperlipidemia in rats. From the result, *Erythrina indica* plant was found to possess antihyperlipidemic activity.[32]

Kamalraj R.., conducted a study to evaluate antihyperlipidemic activity of the leaves of *Erythrina indica* Lam in albino rats in which the plants were subjected to successive extraction with aqueous solvent using high fat diet induced hyperlipidemia of rats model. The effect was assessed by parameters like TC, TG, LDL, VLDL and HDL levels. The results are compared to that of standard drug, simvastatin 5mg/kg. So the present study supports the earlier claims of the plant in obesity.[33]

A study was conducted by Sakat Sachin S *et al*., with methanolic extract of *Erythrina indica* Lam leaves to evaluate antiulcer activity in pylorus ligated and indomethacin induced ulceration in albino rats. The effect was assessed by parameters like total acidity, free acidity, peptic activity and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for indomethacin induced ulcer model. From the result, the methanol extract of *E. indica* leaves possessed significant antiulcer properties in a dose dependent manner.[34]

Sakat SS *et al.,* studied the *Erythrina indica* Lam., leaves extracts for antioxidant activity. Aqueous and methanol extracts of *Erythrina indica* leaves were used for *in vitro* methods viz. 1, 1-Diphenyl-2-Picrylhydrazyl, nitric oxide radical scavenging activity, and inhibition of lipid peroxidation by thiobarbituric acid reactive substances (TBARS)

method on isolated rat liver tissues. From the results, it is concluded that the aqueous and methanol extracts of *E. indica* leaves possesses significant antioxidant activity.[35]

Patil D D *et al*., demonstrated with aqueous and methanol extracts of *Erythrina indica* root and bark to evaluate diuretic activity in rats. The effect was assessed by measuring urine volume, sodium and potassium content and chloride content. From the result, aqueous and methanol extracts of *Erythrina indica* produced significant diuretic effect.[36]

Jesupillai M *et al*., studied the leaves of *Erythrina indica* using various extracts of ethanol, chloroform and ethyl acetate for its anthelmintic property against Pheritima Posthuma. The activity was assessed by the determination of time of paralysis and time of death of earth worms. Piperazine citrate (10mg/kg) was included as standard. All the three extracts exhibited good anthelmintic activity.[37]

3.4 PLANT PROFILE

Erythrina indica (Lam.,) belongs to the family of fabaceae.

SYNONYMS

Erythrina corallodendrum var. orientalis L.,

Erythrina indica Lam.

Erythrina orientalis (L.,) Merrill

Tetradapa javanorum Osbeck

BOTANICAL CLASSIFICATION

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Papilionaceae
Genus	:	*Erythrina*
Species	:	*Erythrina indica*

BOTANICAL NAME

Erythrina indica Lam

VERNACULAR NAME[38]

English	:	Indian coral tree
Hindi	:	Pangri, Dadak
Bengali	:	Palte, Madar
Malay	:	Dedap
Tamil	:	kalyanamurungai
Marathi	:	Pangara
Sanskrit	:	Paribhadra

PLANT PICTURE

Fig.4: *Erythrina Indica* whole plant and *E. Indica* stem bark

DISTRIBUTION

Erythrina indica (Coral tree) which is found in the wild tropics and deciduous forests of India.[39]

DESCRIPTION

Erythrina indica Lam. (Family: Fabaceae), commonly known as 'Indian Coral tree' is a medium sized, spiny, deciduous tree with brilliant red blossoms flower, normally grows up to 6–9 m height. It is an evergreen forest tree that grows in both acid and alkaline soils[40,41,42].Young stems and branches are thickly armed with stout conical spines up to 8 mm long, which fall off after 2-4 years; rarely, a few spines persist and are retained with the corky bark. Bark smooth and green when young, exfoliating in papery flakes, becoming thick, corky and deeply fissured with age. Leaves trifoliate, alternate, bright emerald - green, on long petioles 6-15 cm, rachis 5-30 cm long, prickly. Leaf petiole and rachis are spiny. Flowers in bright pink to scarlet erect terminal racemes.

Fruit a cylindrical torulose pod, green, turning black and wrinkly as they ripen, thin-walled and constricted around the seeds. There are 1-8 smooth, oblong, dark red to almost black seeds per pod[43, 44]. Fruits are cylindrical torulose pods, containing 1-8 dark black coloured seeds. *Erythrina* comes from the Greek word *'eruthros'* means red, which is colour of its flowers.[45, 46]

PHYTOCONSTITUENTS

The plant contains alkaloids, flavonoids, sterols, terpenoids, fatty acids and saponins.

Bark and leaves

- Flavonoids such as erytrinin C, 8-prenyl, Indicanin E, isoflavones such as genistein, indicanin D and wighteone.
- Triterpenes such as erythrodiol, oleanlic acid.[47]
- Steroidal compounds such as stigmasterol, β, γ and δ-sitosterol.[48]

Root bark

- Coumarins such as indicanine A and indicanine B.
- Flavonoids such as indicanine C and alpinium isoflavone, 4-5-Di-O-Methyl isoflavone namely cajanin daidzein[49] and 8-prenyl diadzein.[50]

Flowers

- kaempferol.[51]
- Flavones, 4-5-7-trihydroxy-3-methoxy-8-c-prenyl, 7-o-beta-D-glucopyranoyl (1-3) - alpha-7-arabinopyranoside.

Seed

- Seed oil shows presence of linoeic acid, lignoceric acid and oleic acid.[52]
- Dried seed shows presence of lectin.[53]

USES

Leaves

Rheumatic joints, antidote to strychnine poisoning, irregular menstruation and for enhancing milk secretion by lactating mothers[54], gallstone colic, expectorant, febrifuge, and vermifuge[55], fresh leaves are cooked in coconut milk or goat's milk and honey and

used as a health tonic by lactating women for promoting milk secretion and delay premature labour.[56]

Bark

Fever, malaria, rheumatism, toothache, also for boils, antidote to strychnine poisoning, fractures[57,58], anthelmintic, aphrodisiac, laxative, diuretic, emmenagogue[59], The decoction of the bark is reported to be used orally as anthelmintic, expectorant[60], regulate menstrual flow and irregular menstruation, astringent, collyrium in ophthalmia and as antibiliousness agent.[61,62]

Roots

GIT problems, malaria and burns[63] and galactagogue.[64]

Shoot

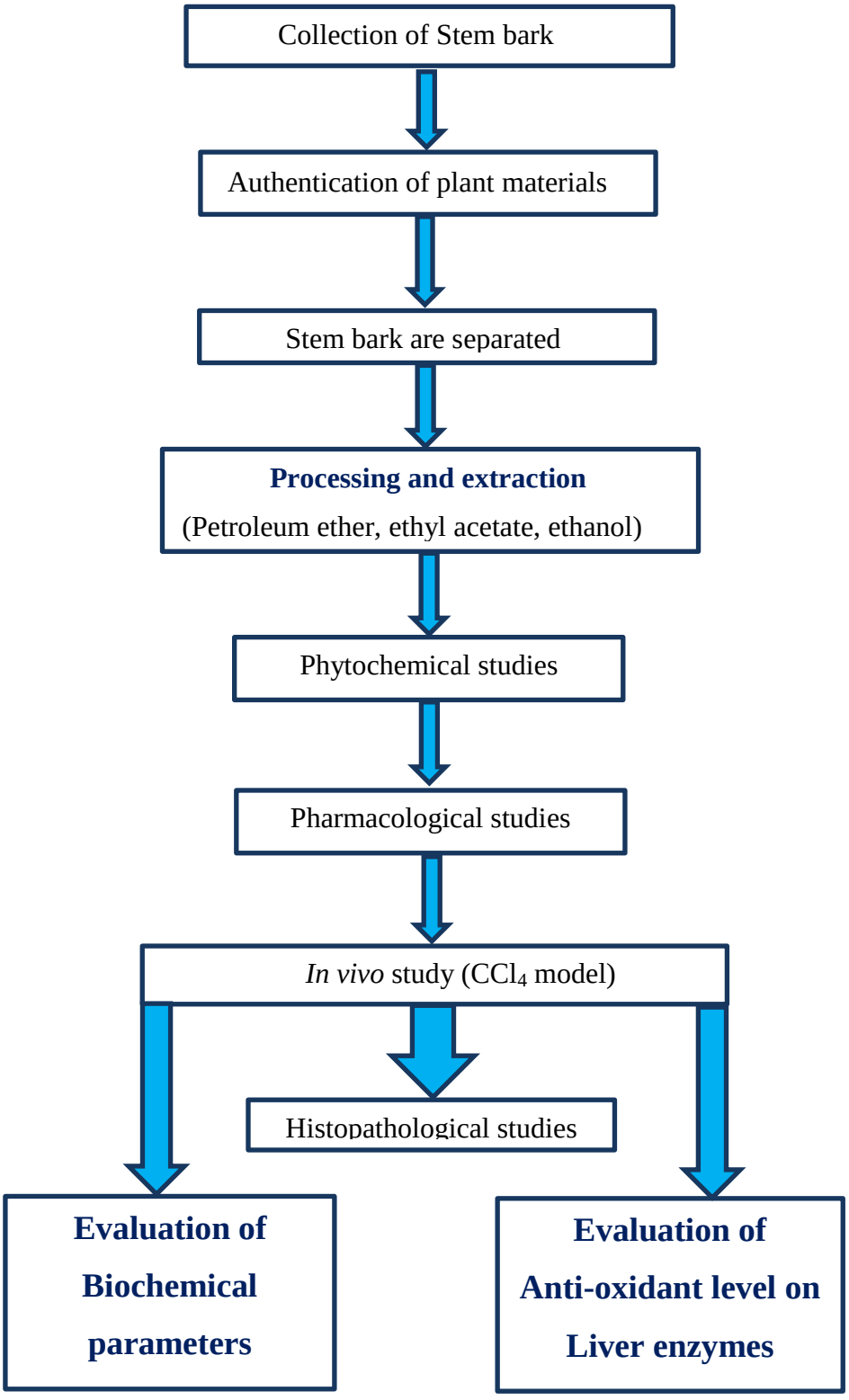
Anthelmintic, aphrodisiac, laxative, diuretic and emmenagogue.[36,37]

SCOPE

&

PLAN OF WORK

4. SCOPE AND PLAN OF WORK



MATERIALS & METHODS

5. MATERIALS AND METHODS

5.1 Plant collection and identification

Fresh stem bark of *Erythrina indica* Lam was collected from the forest around Vellimalai estate, Theni district, Tamilnadu, India in the month of August 2016. It was authenticated by Prof. P. Jayaraman, Director, Retd, Professor, Institute of Herbal Botany, Presidency College, Chennai-5

5.2 Preparation of plant extract[23]

The freshly collected stem bark of this plant was shopped and dried. The dried material was powder. The powdered plant material (250 g) was extracted by hot continuous soxhlet extraction method and the plant material was extracted with Ethanol (99.9% v/v), Ethyl acetate and Petroleum ether for four days in a soxhlet apparatus.

It is a process of continuous extraction method in which the solvent can be circulated though the extractor for several time. The vapours from the solvents are taken to the condenser and the condensed liquid is returned to the extract for continuous extraction. The apparatus consist of body extractor (thimble) attached with side siphon tube fixed in lower and attached with distillation flask and the mouth of the extractor is fixed to the condenser by the standard joints.

Procedure

- Weigh about 250 g dried powdered plant and transferred into a thimble for packing.
- While packing, the content was wetted with Ethanol, Ethyl acetate and Petroleum ether respectively and poured until the siphon tube was filled.

- A piece of porcelain was added into the round bottom flask to avoid bumping effect.
- After assembling the extractor, the plant material was extracted at about (40-45ºC), (35-40ºC) and (20-30ºC) temperature respectively until the colour of the solution in the siphon tube become pale.
- The extracts obtained were dried at room temperature and the yield was stored in air tight container.

DRUG AND CHEMICALS

Ethanol, Ethyl acetate, Petroleum ether, Silymarin, Beta cyclo dextrin,

5.3 PHYTOCHEMICAL ANALYSIS[65]

1. TEST FOR ALKALOIDS

A small portion of the extract was stired with few drops of dilute hydrochloric acid and filtered. The filtrated portion was tested carefully with various alkaloidal reagents such as ***Mayer's reagent*** (cream precipitate), ***Dragendroff's reagent*** (orange brown precipitate), ***Hager's reagent*** (yellow precipitate), and ***Wagner's reagent*** (reddish brown precipitate).

2. TEST FOR FLAVONOIDS

Schinado's test: To the extract, a few manganesium turnings and few drops of concentrated hydrochloric acid were added and boiled for 5 minutes. The red colour indicates the presence of flavonoids.

***Sodium hydroxide test*:** To the extract in ethanol, 10% sodium hydroxide was added. The dark yellow colorations indicate the presence of flavonoids.

3. TEST FOR TANNINS AND PHENOLIC COMPOUNDS

The extract was dissolved in distilled water. The extract was then divided into three parts. A sodium chloride solution (10%) was added to one portion of test's extract, 1% gelatin solution to second portion and the gelatin salt reagent to third portion. Precipitation with the later reagent or with both the gelatin salt reagent was indicative of the presence of tannins. Precipitations of sodium chloride solution indicate a false-positive test. Positive tests were confirmed by the addition of a few drops of dilute ferric chloride (1% ferric chloride) to test extracts, which have black or green coloration.

The extract was mixed with lead acetate solution and observed for white precipitate.

4. TEST FOR TERPENOIDS

***Noller's test*:** The extract was warmed with tin and thionyl chloride. The pink coloration indicates the presence of terpenoids.

5. TEST FOR GLYCOSIDES

***Borntrager's test*:** A small amount of extract was hydrolysed with hydrochloric acid for few hours on a waterbath and the hydrolysate was extracted with benzene. The benzene layer was treated with dilute solution and observed for formation of reddish pink color.

***Legal's test*:** The extract was dissolved in pyridine and was made alkaline with few drops of 10% sodium hydroxide and freshly prepared sodium nitroprusside was added and observed for the formation of blue color.

6. TEST FOR SAPONINS

***Foam test*:** A small amount of extract was extracted with petroleum ether. The insoluble residue left after extraction, a few (ml) of water was added and shaken vigorously for 15 minutes and was observed for the formation of honeycomb forth that persisted for at least 30 minutes.

7. TEST FOR PROTEIN

***Biuret test*:** The extract is treated with an equal volume of 1% strong NaOH following by a few drops of copper (II) sulphate. The formation of purple colour indicates the presence of protein.

***Million's test*:** Million's reagent is added to the extract, a white precipitate is produced, while heating it turns brick red colour. It indicates the presence of protein.

8. TEST FOR MUCILLAGE

The extract is treated with aqueous potassium hydroxide; swelling indicates the presence of mucillage.

9. TEST FOR CARBOHYDRATES

***Molish's reagent* :** To the extract few drops of α-naphthol solution in alcohol, con.H_2SO_4 is added at the side of test tube. The formation of violet ring at the junction of two liquids indicates the presence of carbohydrates.

10. TEST FOR PHYTOSTEROLS

***Libermann-Burchard test*:** 1 mg of the extract was dissolved in few drops of chloroform, 3 ml of acedic anhydride and 3 ml of glacial acedic acid. It is warmed and cooled under tap water and drops of concentrated sulphuric acid were added along the side of the test tube. The formation of bluish green colour indicates the presence of steroids.

FLAVONOID CONTENT[66,67]

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.30 ml of 5% NaNO2, after 5min 0.3 ml of 10 % AlCl3 was added. After 5min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

5.4 *IN VIVO* STUDIES

Experimental Animals

The present study was conducted after obtained approval from the Institutional Animal Ethics Committee, Madras Medical College, Chennai-03. The protocol met the requirements of national guidelines of CPCSEA (**PROPOSAL NO: IAEC/MMC/16/2016**) The Wistar rats (150-200g) used for this study were procured from, Central Animal House, Madras Medical College, Chennai-03.

Quarantine and Acclimatization[68]

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. The newly procured "Wistar Albino rats" were quarantined for the period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop the physiological and nutritional stabilization before their use.

Housing

The animals are housed in well ventilated animal house which is maintained at a constant temperature and relative humidity of 55-65%. The animals are housed in spacious polypropylene cage and paddy husk are utilized as bedding materials.

Diet and water

The animals are maintained and given on standard pellet diet and purified water. The animals are provided with food and water (*ad libitum*) expect during fasting. The bedding materials are changed frequently.

Drug administration

Drug is administered by oral gavage using oral feeding tube fixed to a syringe needle to administer the required quantity of the drug.

Animal identification

All the animal cages used in the study had a proper identification i.e. labels. Each animal in cage was marked either on head or body or tail with picric acid for their appropriate identification.

5.4.1 *IN VIVO* HEPATOPROTECTIVE EVALUATION

The hepatoprotective activity of *Erythrina indica* Lam., was evaluated in Wistar rats. Liver toxicity was induced by intraperitoneal administration of carbon tetrachloride (CCl_4). The hepatoprotective effect of plant extract was compared with standard drug Silymarin.

Drugs and chemicals

- Carbon tetrachloride
- Silymarin
- β - cyclo dextrin (BCD)[69]

Preparation of drug solutions

- Carbon tetrachloride was dissolved in olive oil 1:1
- Extracts was dissolved in 1% BCD
- Standard drug (Silymarin) was dissolved in distilled water.

Experimental method

Carbon tetrachloride induced hepatotoxicity in Wistar albino rats was used as a model for screening for hepatoprotective activity.

5.4.1.1 Acute toxicity study (425)[24,70]

Acute toxicity studies have been already done and it was found to be safe upto 4000mg/kg p.o. Hence, the doses selected for this study were 400 mg/kg p.o.

Carbon tetrachloride induced hepatotoxicity model[70,71]

After a week of acclimatization, the rats were divided into seven groups containing six animals in each.

Table – 2: *In Vivo* hepatoprotective experimental design

S.no	Groups	Treatment schedule	No. of animals
1	**Group I** Normal control	1 ml of 1% BCD p.o. daily for 14 days	**6**
2	**Group II** Negative control	1 mg/kg CCl_4 in olive oil (1:1), i.p. once in 3 days for 14 days and received daily a single oral dose of BCD (1ml of 1% w/v)	**6**
3	**Group III** Positive control	25 mg/kg of Silymarin p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	**6**
4	**Group IV** Test group 1	400mg/kg Ethanolic extract of *Erythrina indica* in BCD p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	**6**
5	**Group V** Test group 2	400 mg/kg Ethyl acetate extract of *Erythrina indica* in BCD p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	**6**
6	**Group VI** Test group 3	400 mg/kg Petroleum ether extract of *Erythrina indica* in BCD p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	**6**
TOTAL ANIMALS			**36**

For all rats, body weight was measured before and after the induction of hepatotoxicity (1^{th} and 15^{th} days). On the 15^{th} day, all the animals were mildly anesthetized and blood was collected by heart puncture and serum was separated by

centrifugation at 2000 rpm for 15-20 minutes at 4^{o}C, the serum samples were maintained at -80^{o} C, for estimation of biochemical parameters.

The animals were sacrificed by cervical dislocation method. The liver is removed and rinsed with ice cold saline and stored in 10% formalin solution. A part of liver was homogenate with phosphate buffer, PH 7.4 using a Teflon homogenizer in ice-cold condition. The homogenate of liver as centrifuged at 5000 rpm for 10 min, the supernatants solution are taken up for the evaluation of lipid peroxidation (LPO), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The other part of liver was subjected to Histopathological study.

5.5 BIOCHEMICAL PARAMETERS

The blood samples were collected and allowed to clot and centrifuged at 2000 rpm for 15-20 minutes using REMI (412 LAG) cooling centrifuge. The serum was kept at -80ºC until analyzed. Levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Serum Alkaline Phosphate (ALP) Total protein, Albumin and Total Bilirubin were determined with an Automated Analyzer (Hitachi 911, Japan).

5.5.1. Estimation of Aspartate Aminotransferase (AST/SGOT)[72,73]

Aspartate Aminotransferase (AST) also referred to as Serum glutamate oxaloacetate transferase (SGOT), is an enzyme involved in amino acids metabolism, AST is widely distributed in liver, RBC, heart, pancreas and kidney. A high level of AST in blood is observed in severe liver disease, kidney disease and lung disease.

Principle

α- Ketoglutarate + L- Aspartate $\xrightarrow{\text{SGOT}}$ **L- Glutamate + Oxaloacetate**

Oxaloacetate + NADH+H^{+} $\xrightarrow{\text{MDH}}$ **L- Malate + NAD^{+}**

The reagent of NADH consumption is measured photometrically and is directly proportional to the AST concentration in the sample.

Reagents

- L- Aspartate 200 mmol/l
- Malate dehydrogenase 200 mmol/l
- α- Ketoglutarate 35 mmol/l
- NaOH 1.05 mmol/l

Procedure

800 µl of L- Aspartate and Malate dehydrogenase and 200 µl of α- Ketoglutarate are mixed together and incubated at 37ºC for 2 minutes and 100 µl of sample is added. The change in absorbance is measured at 340 nm.

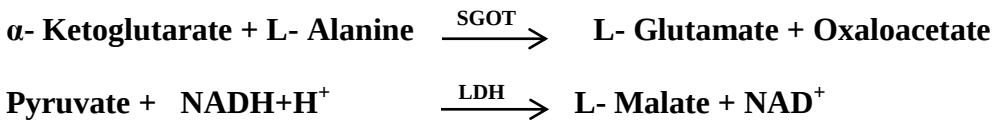
Calculation

AST = Abs/min × 1764 (factor)

5.5.2. Estimation of alanine aminotransferase (ALT/SGPT)[73]

Alanine aminotransferase / Serum Glutamate Pyruvate Transferase are an enzyme which is involved in amino acids metabolism.

Principle



The rate of NADH consumption is measured photometrically and is directly proportional to the ALT concentration in the sample.

Reagents

- L- Alanine 200 mmol/l
- Lactate dehydrogenase 200 mmol/l
- α- Ketoglutarate 35 mmol/l
- NADH 1.05 mmol/l
- Tris buffer 80 mmol/l pH 7.5

Procedure

800 µl of L- Alanine and Lactate dehydrogenase and 200 µl of α- Ketoglutarate are mixed together and incubated at 37ºC for 2 minutes and 100 µl of sample is added. The change in absorbance is measured at 340 nm.

Calculation

ALT = Abs/min × 1764 (factor)

5.5.3. Estimation of alkaline phosphatase (ALP) [74]

Principle

When the enzyme incubated with p-nitro phenyl phosphate and Tris buffer (pH 9.6) in alkaline condition, inorganic phosphate and p-nitro phenol are formed by the catalytic action of alkaline phosphatase. Amount of p-nitro phenol liberated by the enzyme is measured at 420 nm.

2-aminomethyl-1-propanol + p-nitro phenyl phosphate + H_2O

↓

4-nitro phenol + 2-amino-2-methyl-1-propanol phosphate

Requirements

- p-nitro phenyl phosphate (10 mM)

- Tris-HCL pH 9.6 (80 mM)
- NaOH (0.1 N)

Procedure

1ml of p-nitro phenyl phosphate and 1.5 ml of buffer were added with 100 µl of homogenate. The mixture was incubated at 37ºC for 30 mins. Then the reaction was stopped by addition of 0.1 N NaOH. The absobance of liberated p-nitro phenol was measured at 420 nm.

Calculation

$$\textbf{ALP U/I} = \Delta A / 2764$$

5.5.4. Estimation of total bilirubin (TB)[75]

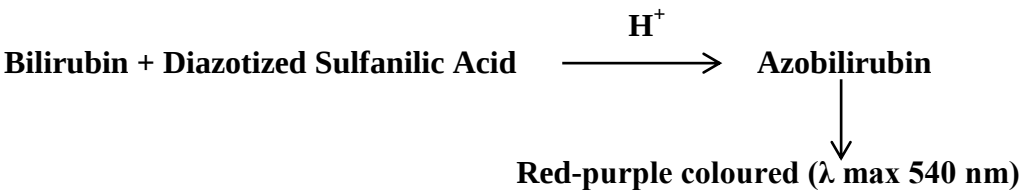
Principle

Method of Malloy and Evelyn used to estimate serum bilirubin level.

Direct : (Conjucated) Bilirubin coupled with diazotization Sulfanilic acid, forming Azobilirubin, a red-purple coloured product in acidic medium.

Indirect : (Unconjucated) Bilirubin coupled with diazotized only in the presence of its dissolving solvent (Methanol). Indirect fraction and thus represents the Total bilirubin concentration. The difference of Total and Direct Bilirubin gives Indirect (Unconjucated) Bilirubin.

The intensity of red-purple colour so developed above is measured calorimetrically and it is proportional to the concentration of the appropriate fraction of Bilirubin. This reaction can be represented as:



Reagents

- Diazo-A, Diazo-B and Diazo blank
- Methanol
- Artificial standard
- Diazo reagent: Just before use, mix 1.0 ml of Diazo-A with 0.03 ml of Diazo-B

Procedure

0.1 ml of serum, 0.25 ml of diazo reagent and methanol 1.25 ml were mixed well and kept in dark at room temperature for 30 mins and read the optical density (O.D) at 540 nm against distilled water on a colorimeter with a yellow green filter.

Calculation

$$\textbf{Total Bilirubin} = \frac{\textbf{O.D.}_{\textbf{T1}}\textbf{-O.D.}_{\textbf{T2}}}{\textbf{O.D. Standard}} \times \textbf{10}$$

5.5.5. Estimation of Total Protein (TP) (Biuret Method)

Serum content of the soluble proteins, those circulating in extracellular and intracellular fluids, has been used as a marker to aid in clinical diagnosis. Serum total protein including albumin is mainly involved in the maintenance of osmotic pressure of plasma and is used to transport many substances including macromolecules.

Principle

In the biuret reaction, a chelate is formed between the Cu^{2+} ion and the peptide bonds of the proteins in alkaline solution to form a violet coloured complex whose absorbance is measured photometrically at 540 nm. The intensity of the colour produced is proportional to the concentration of the protein in the sample.

$$Cu^{2+} + \text{Serum protein} \longrightarrow \text{Copper-protein complex (Violet coloured)}$$

Reagent

- Total protein reagent (Bilirubin reagent)
- Protein standard (Standard prepared from bovine serum albumin)
- Serum sample from animals

Procedure

1ml of biuret reagent which is stored under 2-8ºC and 10 µl of serum sample/standard are mixed well and incubated for 5 mins. The intensity of the colour is measured at 540 nm.

Calculation

$$\text{Total protein in gm/ml} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$$

5.5.6. Estimation of Albumin (ALB)

Principle

Bromocresol-Green (BCG) method

When albumin binds with bromocresol green in a suitable buffer pH 4.15-4.25, an intense blue coloured albumin-BCG complex is formed

$$\text{Albumin + BCG (Yellow)} \longrightarrow \text{Albumin-BCG complex (blue)}$$

Reagent

- Bromocresol green reagent
- Serum sample from animals

Procedure

1ml of bromocresol reagent, 1.5 ml of buffer and 0.2 ml serum were mixed and kept at room temperature for 10 mins. The absorbance was measured at 630 nm.

5.6 ESTIMATION OF LIPID PEROXIDATION, SUPEROXIDE DISMUTASE, GLUTATHIONE PEROXIDASE AND CATALASE LEVELS [77,78]

5.6.1. Estimation of lipid peroxidation (LPO)

The extent of lipid peroxidation was estimated as evidenced by the formation of Thiobarbituric acid reactive substance (TBARS).

TBARS

Lipid peroxidation was estimated by the method of Ohkawa *et al*. (1979). The pink colored chromogen formed by the reaction of 2-thiobarbituric acid (TBA) with breakdown products of lipid peroxidation was read at 532 nm.

Reagents

- 8.1% sodium dodecyl sulphate (SDS)
- 20 % acetic acid.
- 0.5% 2-thiobarbituric acid (TBA)
- N-Butanol and pyridine (15:1 v/v)
- Stock melondialdehyde solution
- 1,1,3,3 - tetramethoxy propone (184 µg/ml)

Procedure

To 0.2 ml of liver homogenate, 0.2 ml of 8.1 % SDS, 1.5 ml of 20 % acetic acid, (adjusted to pH 2 to 3.5 with NaOH) and 1.5 ml of 0.8 % aqueous solution of TBA were

added. The mixture was made up to 4.0 ml with distilled water and then heated in a water bath at 95°C for 60 minutes. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine were added and shaken vigorously. After centrifugation at 4000 g for 10 minutes, the organic layer was removed and its absorbance at 532nm was measured.

5.6.2. Assay of superoxide dismutase (SOD): Kakkar et al. (1984).

The assay of SOD is based on the inhibition of the formation of NADH-phenazinemethosulphate-nitroblue tetrazolium formazon. The color formed at the end of the reaction can be extracted into n-butanol layer and measured at 560 nm.

Reagents

- Sodium pyrophosphate buffer
- Absolute ethanol
- n-butanol
- Phenazine methosulphate (PMS)
- Nitroblue tetrazolium (NBT)
- NADH

Procedure

0.5ml of tissue homogenate was diluted to 1.0 ml water followed by addition of 2.5 ml of ethanol and 1.5 ml of chloroform (chilled reagents were added). This mixture was shaken for 90 sec at 4º C and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate and 0.3 mL of nitroblue tetrazolium, 0.2mL enzyme preparation and water in a total volume of 2.8 mL. The reaction was initiated by the addition of 0.2 mL NADH. The mixture was incubated at 30C for 90 sec and arrested by the addition of 1ml of glacial acetic acid. The reaction

mixture was shaken with 4 mL of n-butanol. The mixture was allowed to stand for 10 min and centrifuged. The intensity of the chromogen in the n-butanol layer was measured at 560 nm in a spectrophotometer (Genesys 10-S, USA). One unit of enzyme activity is defined as the amount of enzyme that gave 50% incubation of NBT reduction in one minute.

SOD activity was expressed as Unit/min/mg of protein

Calculation:

$$\% \text{ Inhibition} = \frac{\text{O.D value of control} - \text{O.D value of test} \times 100}{\text{O.D of control}}$$

Glutathione peroxidase (GPx)

Principle

$$2\,GSH + H_2O_2 \xrightarrow{\text{Glutathione Peroxidase}} GSSG + 2\,H_2O$$

$$GSSG + \text{ß-NADPH} \xrightarrow{\text{Glutathione Reductase}} \text{ß-NADP} + 2\,GSH$$

Abbreviations used

GSH = Glutathione, Reduced Form

GSSG = Glutathione, Oxidized Form

β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

REAGENTS

a) 50 mM Sodium Phosphate Buffer with 0.40 mM Ethylenediaminetetraacetic Acid
(EDTA), pH 7.01 at 25°C.

b) 1.0 mM Sodium Azide Solution (Buffer w/Azide)

c) β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form (ß-NADPH)

d) Glutathione Reductase Enzyme Solution (GR)

e) 200 mM Glutathione, Reduced (GSH)

f) 10.0 mM Sodium Phosphate Buffer with 1.0 mM Dithiothreitol, pH 7.0 at 25EC (Buffer w/DTT)

g) Glutathione Peroxidase Enzyme Solution

h) 0.042% (w/w) Hydrogen Peroxide (H2O2) (**Prepared in Fresh solution**)

PROCEDURE

Prepare a reaction cocktail by pipetting (in millilitres) the following reagents into Reagent C (β-NADPH vial):

Reagent B (Buffer w/Azide)	-	9.20
Reagent D (GR)	-	0.10
Reagent E (GSH)	-	0.05

Mix by inversion and adjust to pH 7.0 at 25°C with 1 M HCl or 1 M NaOH, if necessary. Pipette (in millilitres) the following reagents into suitable cuvettes

	Test	Blank
Reaction Cocktail	3.00	3.00
Reagent F (Buffer w/DTT)	——	0.05
Reagent G (Glutathione Peroxidase)	0.05	——

Mix by inversion and equilibrate to 25°C. Monitor the A340nm until constant, using a suitable thermostated spectrophotometer. Then add:

	Test	Blank
Reagent H (H_2O_2)	0.05	0.05

Immediately mix by inversion and record the decrease in A340nm for approximately 5 minutes. Obtain the A 340nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS

$$\textbf{Units/100 µl enzyme} = \frac{\textbf{(A 340nm/min Test - A340nm/ min Blank)}\times 2\times 4.1\times \textbf{Df}}{\textbf{(6.22) (0.05)}}$$

2 = 2 µmoles of GSH produced per µmole of ß-NADPH oxidized

4.1 = Total volume (in millilitres) of assay

Df = Dilution factor

6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm

0.05 = Volume (in millilitres) of enzyme used

5.6.3. Estimation of catalase (CAT)

The activity of Catalase was determined by the method of Sinha (1979). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined calorimetrically.

Reagents

- Phosphate buffer
- Hydrogen peroxide
- Dichromate–acetic acid reagent: 1:3 ratios of 5% potassium dichromate and glacial acetic acid.
- Standard hydrogen peroxide

Procedure

0.5ml of tissue homogenate was diluted to 1.0 ml water followed by addition of 2.5 ml of ethanol and 1.5 ml of chloroform (chilled reagents were added). This mixture was shaken for 90 sec at 4º C and then centrifuged. The enzyme activity in the supernatant was determined as follows. 0.1 mL of supernatant, 0.9 mL of phosphate buffer and 0.4 mL of hydrogen peroxide was added. The reaction was arrested after 60 seconds by adding 2.0 mL of dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the color developed was read at 620 nm.

Catalase activity was expressed as mole of H_2O_2 consumed/ min/mg of protein.

5.7 HISTOPHATHOLOGICAL STUDIES

The liver from the animals was rinsed in ice cold 0.9% saline and was fixed in 10% formalin embedded in paraffin and cut into 5 µm thick section using a microtome. Sections were mounted on class slide using standard techniques. The sections were stained with Haematoxylin – Eosin and were examined under a microscope using 400 X magnifications and photographed under a light microscope equipped for photography (Olympus CK 40).

STATISTICAL ANALYSIS[79]

All the values were expressed as mean ± SEM. The data was statistically analyzed by one way ANOVA followed by Dunnet's test. One way analysis of variance (ANOVA) was used to correlate the statistical difference between the variables. $P<0.05$ was considered to be significant. Statistical analysis is done by using GraphPad prism.

RESULTS

6. RESULTS

6.1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table.3: Preliminary phytochemical analysis of various extract of *Erythrina indica* Lam.

TEST	Ethanol Extract	Ethyl acetate Extract	Pet. ether Extract
TEST FOR FLAVONOIDS a) Shinado's test b) Sodium hydroxide test	+	+	+
TEST FOR TANNINS With lead acetate	+	+	-
TEST FOR SAPONINS Foam test	+	+	-
TEST FOR TERPENOIDS With tin and thiol chloride	+	+	-
TEST FOR GLYCOSIDES a) Libermann-burchard's test b) Legal's test c) Borntrager's test	-	-	-
TEST FOR PHYTOSTEROLS Libermann test	+	+	+
TEST FOR MUCILAGE Swelling test	-	-	-
TEST FOR PROTEIN a) Biuret test b) Million's test	+	+	+
TEST FOR CARBOHYDRATE Molish's test	+	+	-
TEST FOR ALKALOIDS a) Dragendroff's test b) Mayer's test c) Hager's test d) Wagner's test	+	+	-

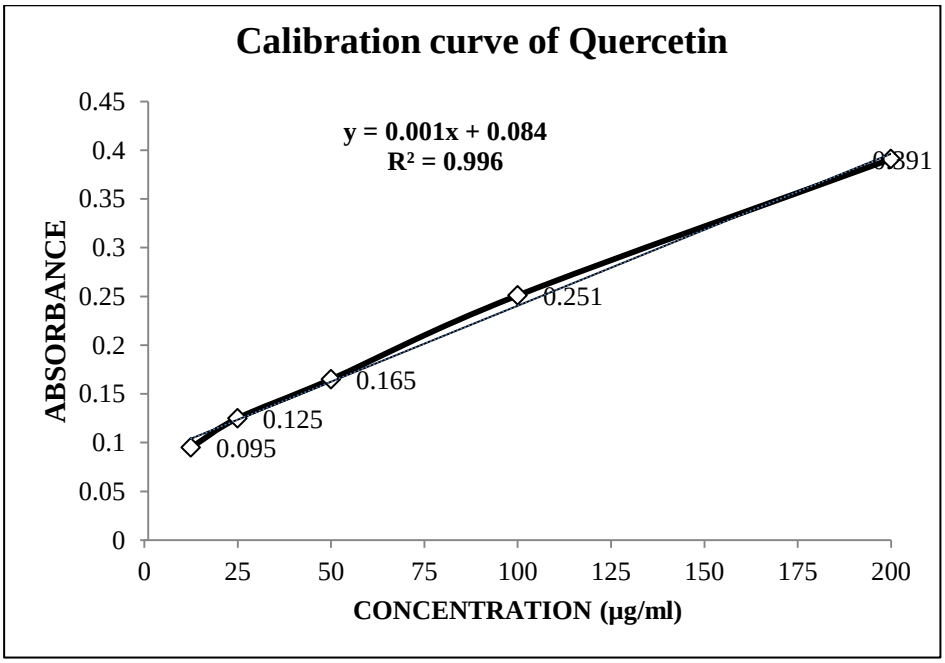
(**+ Present**) (**- absent**)

6.2. DETERMINATION OF TOTAL FLAVONOID CONTENT

Table.4: Standard calibration curve of varying concentration of Quercetin

S. No	Concentration of Quercetin (µg/ml)	Mean Absorbance
1	12.5	0.095
2	25	0.125
3	50	0.165
4	100	0.251
5	200	0.391

Fig.5: Standard calibration curve of varying concentration of Quercetin



The total flavonoid content present in the extracts was determined using aluminium chloride colorimetric method from the calibration curve of standard quercetin. The total flavonoid content in the ethanolic extract of *Erythrina indica* Lam. was found to be 19.67±0.333g QE/100g of extract and ethyl acetate extract of *Erythrina*

indica Lam. was 6±0.577g QE/100g of extract. Petroleum ether extract of *Erythrina indica* Lam. was 13.33±0.333g QE/100g of extract.

Fig.6: Total flavonoid content of ethanol ethyl acetate and petroleum ether extracts of *Erythrina indica* Lam.

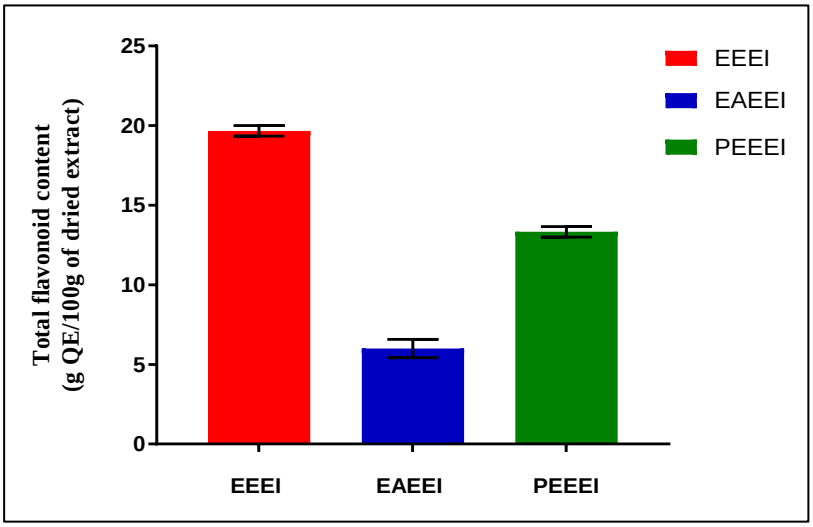


Table.5: Practical yield of *Erythrina indica* Lam

Solvent	Practical yield in percentage
ethanol	10.04% w/w
Ethyl acetate	4.2% w/w
Petroleum ether	2.6% w/w

6.3. *IN VIVO* HEPATOPROTECTIVE ACTIVITY

6.3.1. Body weight

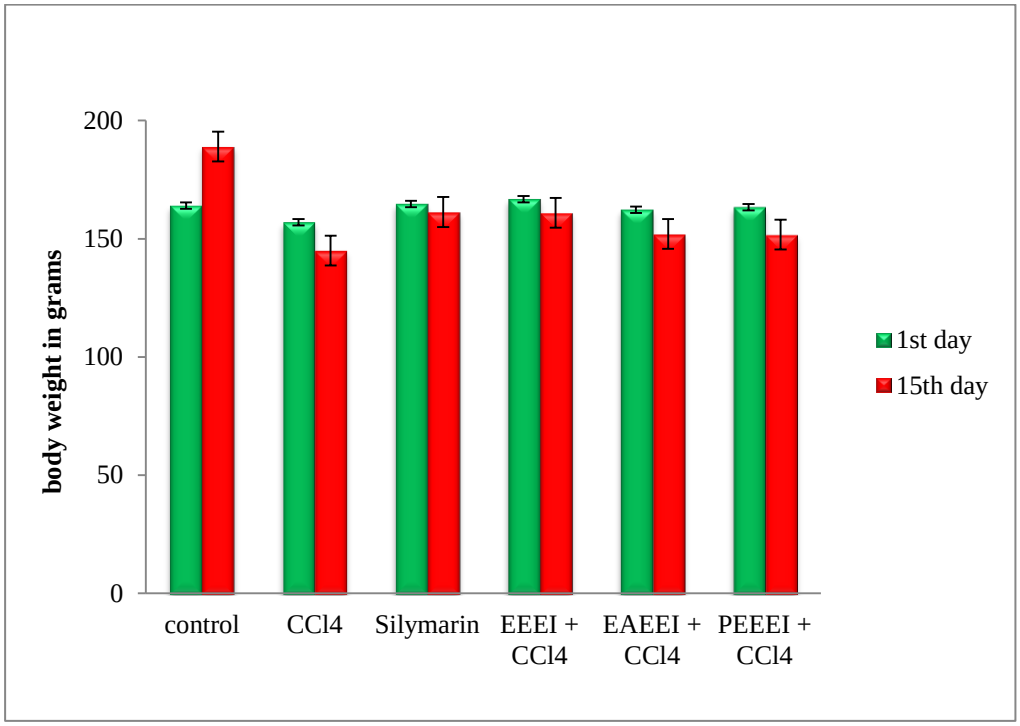
The body weight of the animals was determined on 1st and 15th day of the study period and these are tabulated in **Table-6 and Figure. 7**

Table – 6: Body weight of the animals in the various groups

GROUPS	TREATMENT	ANIMAL BODY WEIGHT IN gms	
		1st day	**15th day**
I	Control	164±3.43	189±2.96
II	Disease control	157±2.31	145±2.73
III	Silymarin (25 mg/kg)	164.7±2.94	161.3±3.18
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	166.7±2.46	161±2.21
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	162.3±2.36	152±1.95
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	163.3±3.82	151.8±2.75

Values are expressed by Mean ± SEM

Fig. 7: Body weight of animals in the various groups



It is seen from the data that in the CCl_4 treated group there was a slight decrease in body weight on the 15th day. In the Silymarin and extracts of *Erythrina indica* Lam treated group shows the reduction in the body weight was lesser than that of CCl_4 treated group.

6.4. BIOCHEMICAL ESTIMATION

6.4.1. Aspartate Aminotransferase (AST) evaluations

The AST level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ Extracts of *Erythrina indica* Lam were estimated on Day 15. They are tabulated in **Table-7 and Figure. 8**

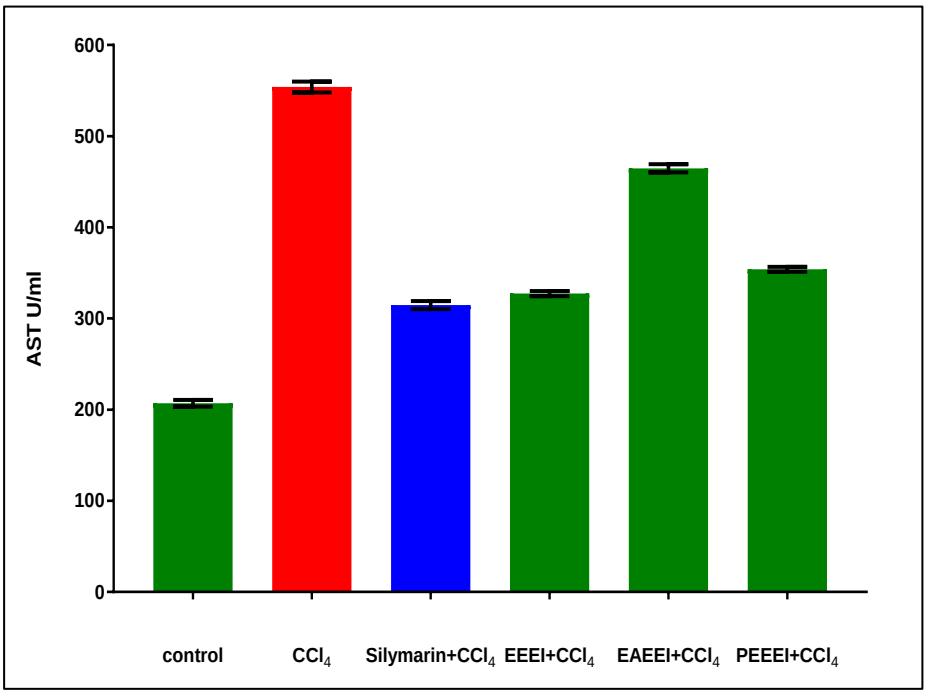
Table – 7: Aspartate Aminotransferase levels

GROUP	TREATMENT	AST (U/ml)
I	Control	207±3.57***
II	Disease control	554±5.96***
III	Silymarin (25 mg/kg)	314.7±4.43***
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	327.3±4.43***
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	464.7±4.55***
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	354±2.75***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group**
###**P<0.001 compared to disease control group**

Fig. 8: Aspartate Aminotransferase levels



In the **Table-7 and Fig.8:** It was seen that AST level 554±5.96 had increased significantly in animals which were given CCl_4 as compared to normal group 207±3.57. Treatment with Silymarin showed a significant decrease in the level of AST 314.7±4.43 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant decrease in the level of AST 327.3±4.43, 464.7±4.55 and 354±2.75 respectively. The reduction was more in the group treated with the ethanolic extracts of *Erythrina indica* 327.3±4.43, when compare to other extracts treated groups.

6.4.2. Alanine aminotransferase (ALT) evaluation

The ALT level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated on day 15. They are tabulated in **Table-8** and **Figure. 9**

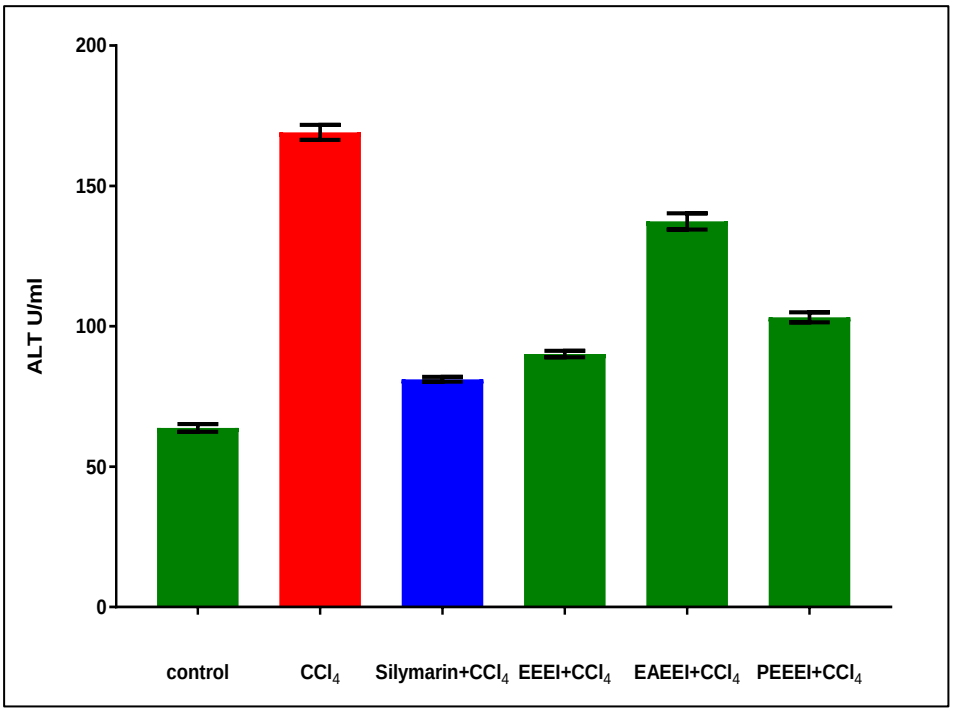
Table – 8: Alanine aminotransferase levels

GROUP	TREATMENT	ALT (U/ml)
I	Control	63.83±1.38***
II	Disease control	169.1±2.69***
III	Silymarin (25 mg/kg)	81.13±0.85***
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	90.16±1.14***
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	137.4±2.89***
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	103.2±1.82***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group**
###**P<0.001 compared to disease control group**

Fig.9: Alanine aminotransferase levels



In the **Table-8** and **Fig.9:** It was seen that ALT level 169.1±2.69 had increased significantly in animals which were given CCl_4 as compared to normal group 63.83±1.38. Treatment with Silymarin showed a significant decrease in the level of ALT 81.13±0.85 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant decrease in the level of ALT 90.16±1.14, 137.4±2.89 and 103.2±1.82 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Erythrina indica* 90.16±1.14, when compare to other extracts treated groups.

6.4.3. Alkaline phosphatase (ALP) evaluation

The ALP level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated on day 15. They are tabulated in **Table-9** and **Figure. 10**

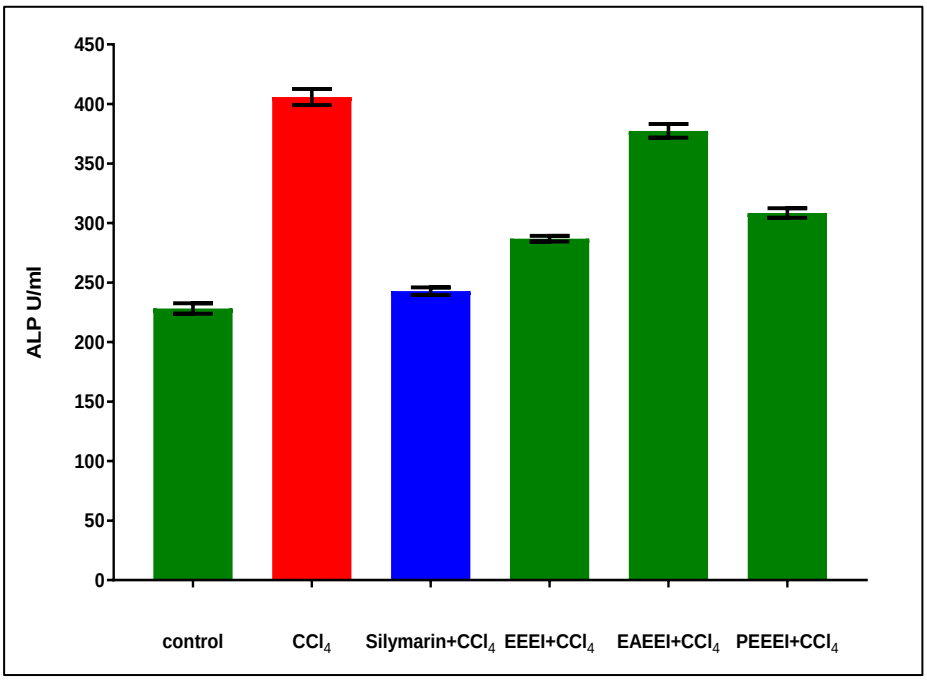
Table – 9: Alkaline phosphatase levels

GROUP	TREATMENT	ALP (U/ml)
I	Control	228.2±4.32***
II	Disease control	405.9±6.76***
III	Silymarin (25 mg/kg)	242.8±3.18***
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	286.9±2.41***
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	377.4±5.75#
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	308.4±4.002***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group**
#P<0.001 compared to disease control group

Fig.10: Alkaline Phosphatase levels



In the **Table-9** and **Fig.10:** It was seen that ALP level 405.9±6.76 had increased significantly in animals which were given CCl_4 as compared to normal group 228.2±4.32. Treatment with Silymarin showed a significant decrease in the level of ALP 242.8±3.18 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant decrease in the level of ALP 286.9±2.41, 377.4±5.75 and 308.4±4.002 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Erythrina indica* 286.9±2.41, when compare to other extracts treated groups.

6.4.4. Total bilirubin (TB) evaluation

The Total Bilirubin level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated on day 15. They are tabulated in **Table-10** and **Figure. 11**

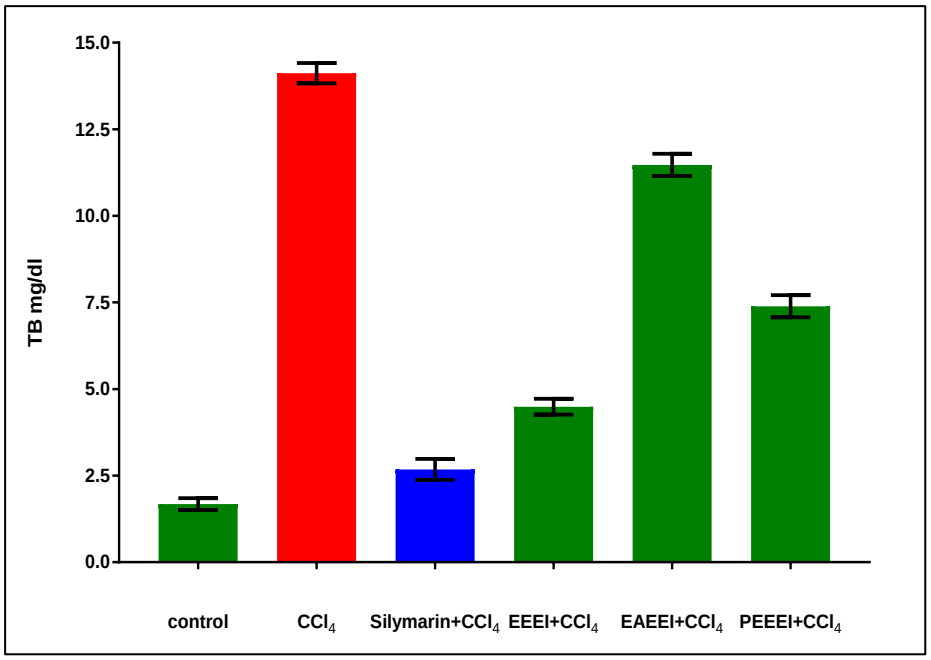
Table – 10: Total bilirubin levels

GROUP	TREATMENT	TB (mg/dl)
I	Control	$1.68\pm0.17^{***}$
II	Disease control	$14.12\pm0.29^{***}$
III	Silymarin (25 mg/kg)	$2.68\pm0.30^{***}$
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	$4.49\pm0.23^{***}$
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	$11.47\pm0.32^{\#\#}$
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	$7.39\pm0.32^{***}$

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group
$^{\#\#}$P<0.001 compared to disease control group

Figure. 11: Total bilirubin levels



In the **Table-10** and **Fig.11:** It was seen that Total Bilirubin level 14.12±0.29 had increased significantly in animals which were given CCl_4 as compared to normal group 1.68±0.17. Treatment with Silymarin showed a significant decrease in the level of Total Bilirubin 2.68±0.30 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant decrease in the level of Total Bilirubin 4.49±0.23, 11.47±0.32 and 7.39±0.32 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups 4.49±0.23.

6.4.5. Total Protein (TP) evaluation

The Total Protein level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated on day 15. They are tabulated in **Table-11** and **Figure. 12**

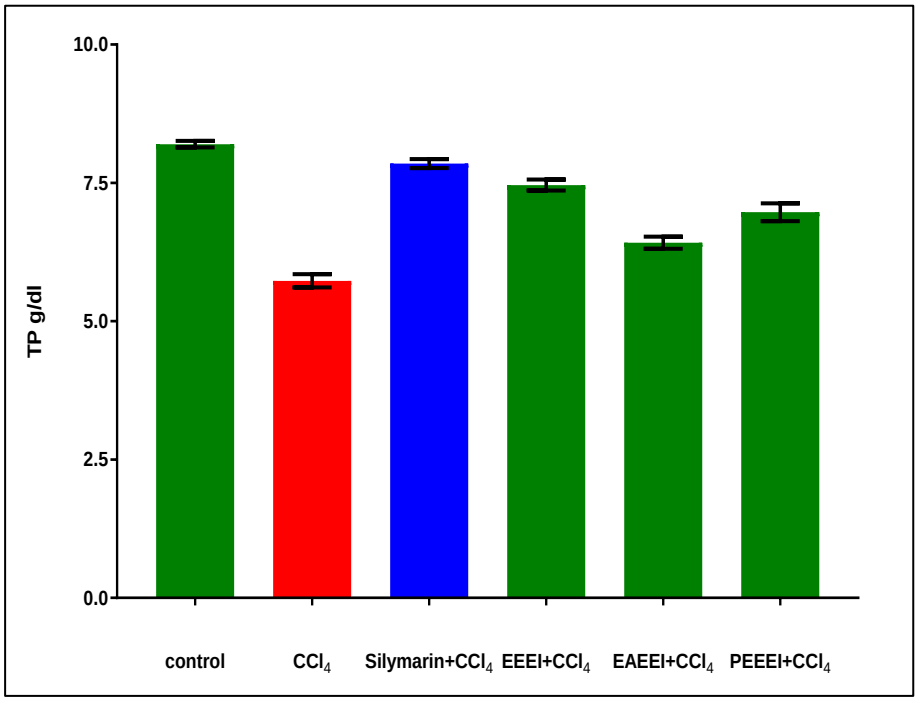
Table – 11: Total Protein levels

GROUP	TREATMENT	TP (g/dl)
I	Control	$8.20\pm0.06^{***}$
II	Disease control	$5.73\pm0.12^{***}$
III	Silymarin (25 mg/kg)	$7.85\pm0.08^{***}$
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	$7.46\pm0.1^{***}$
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	$6.42\pm0.11^{\#}$
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	$6.97\pm0.16^{\#}$

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group
$^{\#}$P<0.001 compared to disease control group

Fig.12: Total Protein levels



In the **Table-11** and **Fig.12:** It was seen that Total Protein level 5.73±0.12 had decreased significantly in animals which were given CCl_4 as compared to normal group 8.20±0.06. Treatment with Silymarin showed a significant increase in the level of Total Protein 7.85±0.08 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant increase in the level of Total Protein 7.46±0.1, 6.42±0.11 and 6.97±0.16 respectively. The elevation was more in the group treated with the Ethanolic extracts of *Erythrina indica* 7.46±0.1, when compare to other extracts treated groups.

6.4.6. Albumin (ALB) evaluation

The Albumin level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated on day 15. They are tabulated in **Table-12** and **Figure. 13**

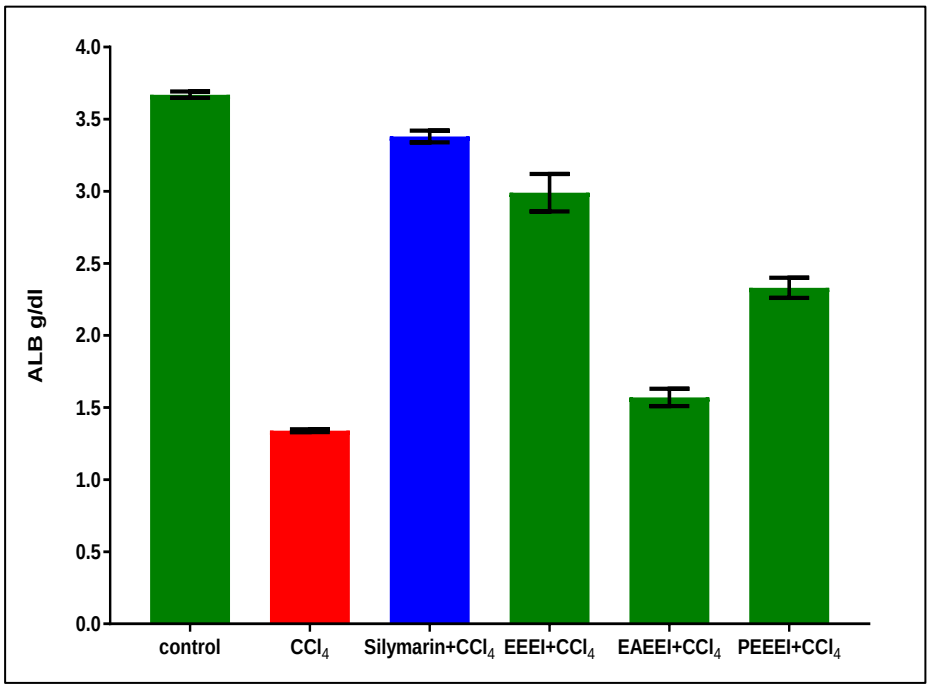
Table – 12: Albumin levels

GROUP	TREATMENT	Albumin (g/dl)
I	Control	3.67±0.02***
II	Disease control	1.34±0.01***
III	Silymarin (25 mg/kg)	3.38±0.04***
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	2.99±0.13***
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	1.57±0.06#
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	2.33±0.07***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group**
#P<0.001 compared to disease control group

Fig.13: Albumin levels



In the **Table-12** and **Fig.13:** It was seen that albumin level 1.34±0.01 had decreased significantly in animals which were given CCl_4 as compared to normal group 3.67±0.02. Treatment with Silymarin showed a significant increase in the level of albumin 3.38±0.04 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant increase in the level of albumin 2.99±0.13, 1.57±0.06 and 2.33±0.07 respectively. The elevation was more in the group treated with the ethanolic extract of *Erythrina indica* 2.99±0.13, when compare to other extracts treated groups.

6.5. ESTIMATION OF ANTIOXIDANT ENZYMES LEVELS

6.5.1. Lipid peroxidation (LPO)

Lipid peroxidase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-13** and **Fig. 14**

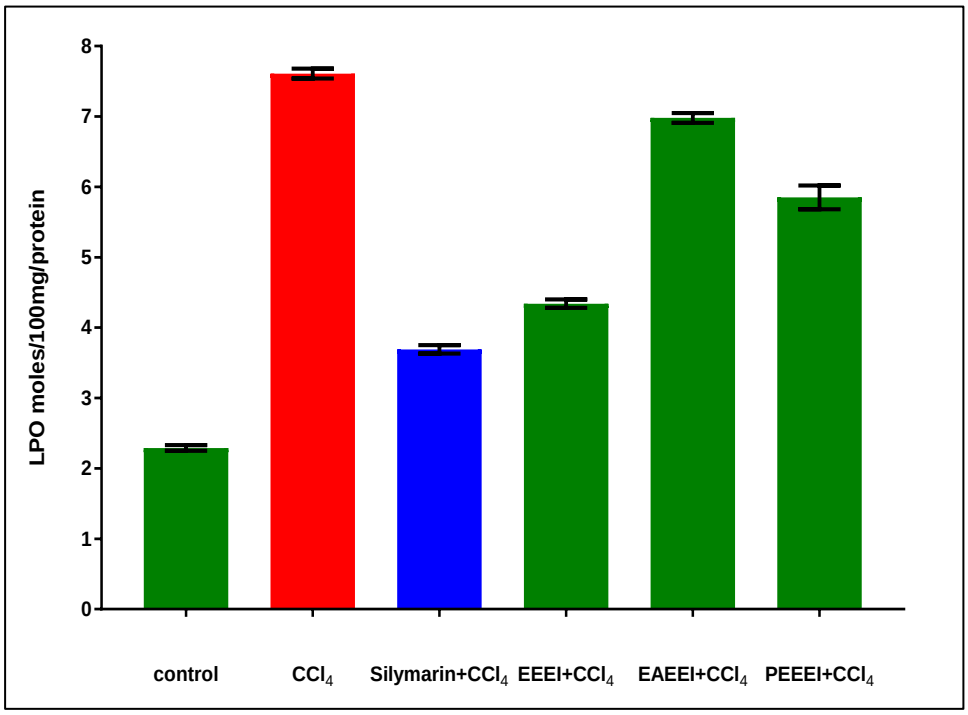
Table – 13: Lipid peroxidation levels

GROUP	TREATMENT	LPO (moles/100mg/protein)
I	Control	2.29±0.04
II	Disease control	7.61±0.07***
III	Silymarin (25 mg/kg)	3.69±0.06***
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	4.34±0.06***
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	6.98±0.07##
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	5.85±0.17##

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group**
##P<0.001 compared to disease control group

Fig.14: Lipid peroxidation levels



In the **Table-13** and **Fig.14:** It was seen that the LPO level 7.61±0.07 had increased significantly in animals which were given CCl_4 as compared to normal group 2.29±0.04. Treatment with Silymarin showed a significant decrease in the level of LPO 3.69±0.06 as compare to the disease control. The extracts *Erythrina indica* Lam treated groups of the animals also showed a significant decrease in the level of LPO 4.34±0.06, 6.98±0.07 and 5.85±0.17 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Erythrina indica* 4.34±0.06, when compare to other extracts treated groups.

6.5.2. Superoxide dismutase (SOD)

Superoxide dismutase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-14** and **Fig. 15**

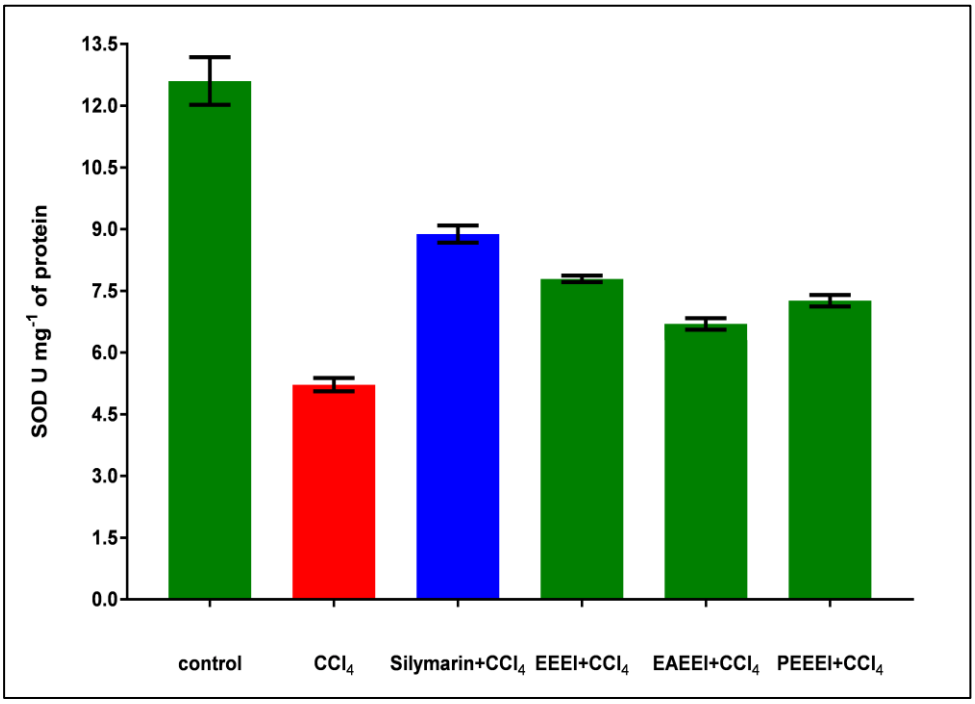
Table – 14: Superoxide dismutase levels

GROUP	TREATMENT	SOD (U mg^{-1} of protein)
I	Control	$12.6\pm0.58^{***}$
II	Disease control	$5.22\pm0.16^{***}$
III	Silymarin (25 mg/kg)	$8.88\pm0.21^{***}$
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	$7.79\pm0.08^{***}$
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	$6.40\pm0.1^{\#\#}$
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	$7.26\pm0.14^{***}$

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group
$^{\#\#}$P<0.001 compared to disease control group

Fig.15: Superoxide dismutase levels



In the **Table-13** and **Fig.15:** It was seen that superoxide dismutase enzyme level 5.22±0.16 had decreased significantly in animals which were given CCl_4 as compared to normal group 12.6±0.58. Treatment with Silymarin showed a significant increase in the level of superoxide dismutase 8.88±0.21 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant increase in the level of superoxide dismutase 7.79±0.08, 6.40±0.1 and 7.26±0.14 respectively. The elevation was more in the group treated with the ethanolic extracts of *Erythrina indica* 7.79±0.08, when compare to other extracts treated groups.

6.5.3. Glutathione peroxidase (GPx)

Glutathione peroxidase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-15**and **Fig.16**

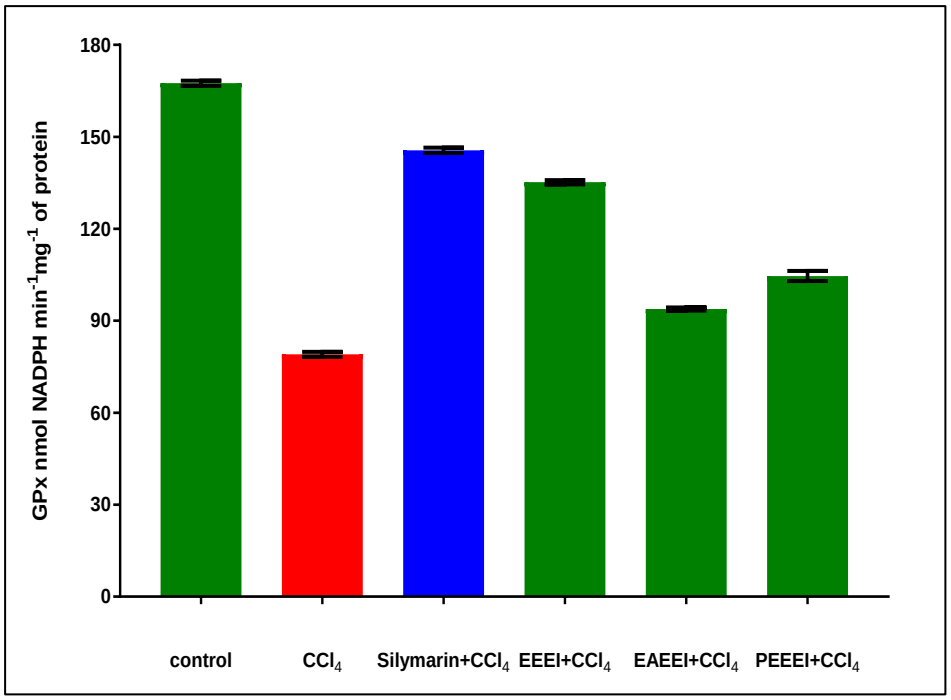
Table –15: Glutathione peroxidase levels

GROUP	TREATMENT	GPx (nmol NADPH min^{-1} mg^{-1} of protein)
I	Control	$167.5\pm0.83^{***}$
II	Disease control	$79.1\pm0.81^{***}$
III	Silymarin (25 mg/kg)	$145.6\pm0.91^{***}$
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	$135.2\pm0.69^{***}$
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	$93.85\pm0.50^{***}$
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	$104.6\pm1.64^{***}$

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group
$^{###}$P<0.001 compared to disease control group

Fig.16: Glutathione peroxidase levels



In the **Table-15** and **Figure.16:** It was seen that Glutathione peroxidase enzyme level 79.1±0.91 had decreased significantly in animals which were given CCl_4 as compared to normal group 167.5±0.83. Treatment with Silymarin showed a significant increase in the level of Glutathione peroxidase 145.6±0.91 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant increase in the level of Glutathione peroxidase 135.2±0.69, 93.85±0.50 and 104.6±1.64 respectively. The elevation was more in the group treated with the Ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups 135.2±0.69.

6.5.4. Catalase (CAT)

Catalase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Erythrina indica* Lam were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-16** and **Fig.17**

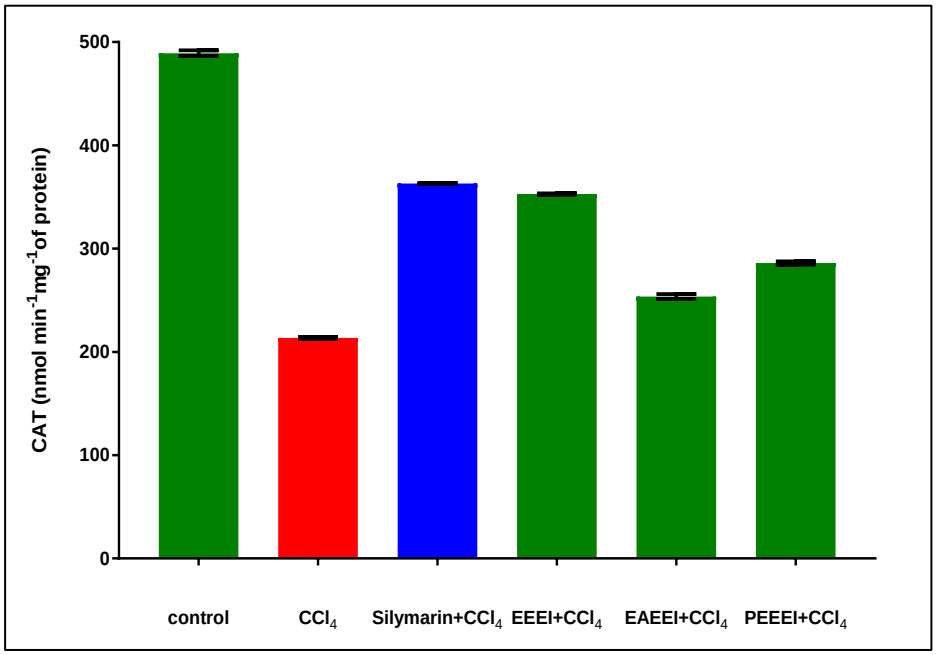
Table – 16: Catalase levels

GROUP	TREATMENT	CATALASE (nmol min^{-1} mg^{-1} of protein)
I	Control	$489.2 \pm 2.75^{***}$
II	Disease control	$213.6 \pm 0.99^{***}$
III	Silymarin (25 mg/kg)	$363.1 \pm 0.54^{***}$
IV	Ethanolic *Erythrina indica* Lam (400 mg/kg)	$352.8 \pm 0.76^{***}$
V	Ethyl acetate extract of *Erythrina indica* Lam (400 mg/kg)	$253.6 \pm 2.38^{***}$
VI	Pet. ether extract of *Erythrina indica* Lam (400 mg/kg)	$286 \pm 1.58^{***}$

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group
$^{###}$P<0.001 compared to disease control group

Fig.17: Catalase levels

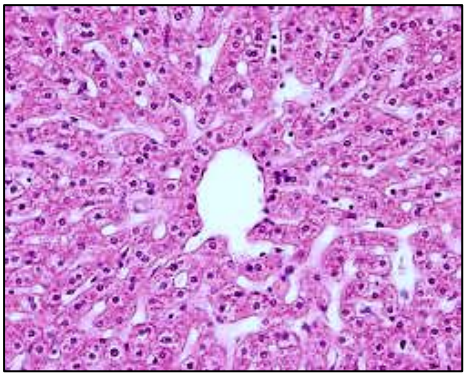


In the **Table-16** and **Fig.17:** It was seen that CAT enzyme level 213.6±0.09 had decreased significantly in animals which were given CCl_4 as compared to normal group 489.2±2.75. Treatment with Silymarin showed a significant increase in the level of CAT 363.1±0.54 as compare to the disease control. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant increase in the level of CAT 352.8±0.76, 253.6±2.38 and 286±1.58 respectively. The elevation was more in the group treated with the ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups 352.8±0.76.

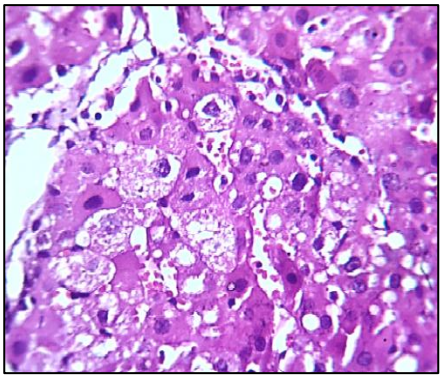
6.6. HISTOPATHOLOGICAL STUDY

A part liver tissue subjected to histopathological evaluation.

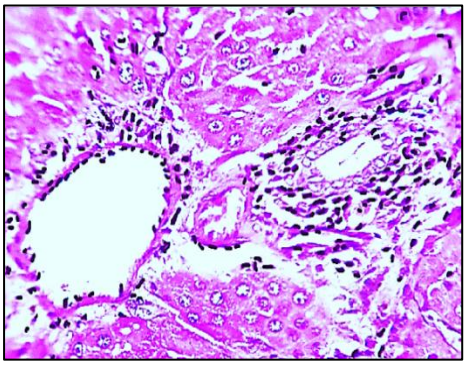
Fig. 18: Histopathological studies of liver



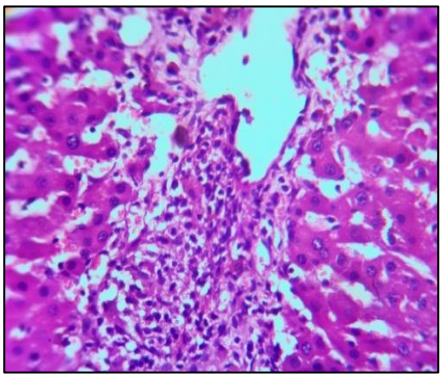
1) Normal control



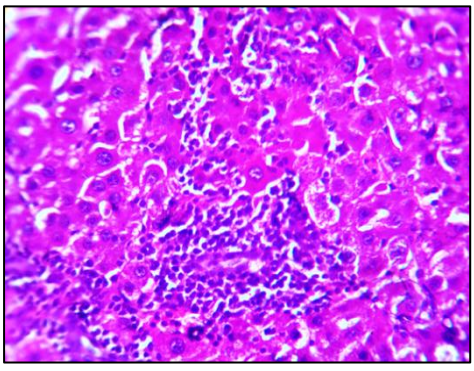
2) CCl_4 treated



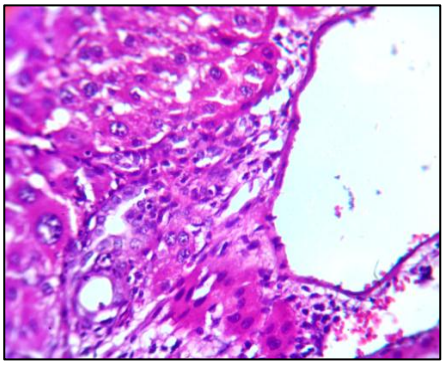
3) CCl_4 + Silymarin 25 mg/kg



4) CCl_4 + EEEI 400 mg/kg



5) CCl_4 + EAEEI 400 mg/kg



6) CCl_4 + PEEEI 400 mg/kg

1. In control group, Liver section showing normal histological appearance.
2. CCl_4 induced group of Liver section showed diffuse areas of vacuolar degeneration, lobular inflammation, portal to portal fibrosis and centrilobular necrosis with mononuclear cell infiltration.
3. Liver section, standard drug silymarin treated group was showing mild hepatocyte vacuolation.
4. Liver section of CCl_4 along with ethanolic extract of *E.indica* 400 mg/kg: treated group was showing mild vacuolar degeneration and mild hepatocyte swelling.
5. Section of CCl_4 along with ethyl acetate extract of *E.indica* 400 mg/kg treated group has shown vacuolar degeneration, lymphocyte present in portal tract and mononuclear cell infiltration in parenchyma and portal areas.
6. Liver section of CCl_4 along with pet ether extract of *E.indica* 400 mg/kg treated group has shown mild vacuolar degeneration and mild hepatocyte swelling.

DISCUSSION

7. DISCUSSION

Liver is an important organ involved in metabolism of many xenobiotics. It removes toxins from the body. It is also exposed to several drugs and xenobiotics which cause hepatic damage. In the present study hepatoprotective activity on the various extracts of stem bark of *Erythrina indica* Lam was evaluated.

The powdered plant material was extracted by hot continuous soxhlet extraction method and the plant material was extracted with Ethanol (99.9% v/v), Ethyl acetate and Petroleum ether for four days in a soxhlet apparatus. The practical yield of ethanolic extract showed 10.04% w/w, ethyl acetate extract showed 4.2% w/w and petroleum ether extract showed 2.6% w/w.

In phytochemical evaluation of ethanolic extract of stem bark of *Erythrina indica* Lam showed the presence of flavonoids, tannins, saponins, terpenoids, phytosterol, protein, carbohydrate and alkaloids. Ethyl acetate extract of stem bark of *Erythrina indica* Lam showed the presence of flavonoids, tannins, saponins, terpenoids, phytosterol, protein, carbohydrate and alkaloids. Petroleum ether extract of stem bark of *Erythrina indica* Lam showed the presence of flavonoids, phytosterol and protein.

It was seen that ethanolic extract of stem bark of *Erythrina indica* Lam showed increased total flavonoid content when compared to other extracts. The observed *in vivo* antioxidant and hepatoprotective activity for this extract therefore may be due to the presence of flavonoids[80,81]. The acute toxicity test suggested that the crude extracts of the plant was non-toxic to rat upto the dose 4000 mg/ kg.

CCl_4- induced hepatic injury is an experimental model widely used for the screening of hepatoprotective drugs. CCl_4 undergoes a biotransformation by hepatic

microsomal cytochrome P-450 to produce trichloromethyl free radicals. This hepatotoxic metabolite can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes. As a result of hepatic injury, permeability of the cell membrane is altered causing the cytosolic transaminase (ALT, AST), in the circulation. Hence evaluation of AST and ALT are definite indicators of hepatoprotective activity. The rise in the serum levels of ALP, AST, ALT and bilirubin as observed in the present study could be attributed to the damaged structural integrity of the liver. Liver damage is also associated with elevated levels of ALT, and Bilirubin. It is also associated with decrease in levels of Total Protein and Albumin.[82]

It was seen that AST, ALT, ALP and bilirubin levels had increased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant decrease in the level of AST, ALT, ALP and bilirubin. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant decrease in the level of AST, ALT, ALP and bilirubin. The reduction was more in the group treated with the Ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups.

Total Protein, Albumin levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the level of Total Protein, Albumin. The extracts of *Erythrina indica* Lam treated groups of animals also showed a significant increase in the level of Total Protein, Albumin. The elevation was more in the group treated with the ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups.

The administration of various extracts of stem bark of *Erythrina indica* Lam showed improvement in the biochemical parameters profile of the animals. The effect

was seen with ethanolic extract of stem bark of *E. indica* is almost equal to that of the standard drug Silymarin. These evaluation studies confirm the hepatoprotective potential of various extracts of stem bark of *Erythrina indica* Lam.

It has been hypothesized that one of the principle causes of CCl_4- induced liver injury is formation of lipid peroxidases by free radical derivatives of CCl_4 (CCl_3^-). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4- induced hepatotoxicity.

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl_4 and is responsible for the cell membrane alteration. In the present study, elevated level of LPO observed in CCl_4 administered rats indicated excessive formation of free radicals and activation of LPO system resulting in hepatic damage[81]. It was seen that the LPO levels had increased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant decrease in the level of LPO. The various extracts of stem bark of *Erythrina indica* Lam treated groups of the animals also showed a significant decrease in the levels of LPO. The reduction was more in the group treated with the ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups. Hence, it is possible that the mechanism of hepatoprotection of *Erythrina indica* Lam might be due to its antioxidant action.

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, Glutathione peroxidase and Catalase. SOD has been reported as one of the most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the

toxic effect caused by this radical. Decrease in enzymatic activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury[83]. It was seen that Superoxide dismutase enzyme levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the level of Superoxide dismutase. The various extracts of stem bark of *Erythrina indica* Lam causes a significant increase in hepatic SOD level indicating a reduction of reactive free radical induced oxidative damage to liver. The elevation was more in the group treated with the ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water[84,85]. It was seen that Glutathione peroxidase enzyme levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the levels of Glutathione peroxidase. The various extracts of stem bark of *Erythrina indica* Lam causes a significant increase in hepatic GPx level indicating a reduction of reactive free radical induced oxidative damage to liver. The elevation was more in the group treated with the ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) and protects the tissues from highly reactive hydroxyl radicals[83]. Therefore reduction in the level of CAT may result in a number of deleterious effects due to the assimilation of

superoxide radical and hydrogen peroxide[85,86]. It was seen that catalase enzyme levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the levels of catalase. The various extracts of stem bark of *Erythrina indica* Lam causes a significant increase in hepatic Catalase level indicating a reduction of reactive free radical induced oxidative damage to liver. The elevation was more in the group treated with the ethanolic extracts of *Erythrina indica*, when compare to other extracts treated groups.

From the control group, Liver section showing normal histological appearance. CCl_4 induced group of Liver section showed diffuse areas of vacuolar degeneration, lobular inflammation, portal to portal fibrosis and centrilobular necrosis with mononuclear cell infiltration. The liver section of standard drug silymarin treated group was showing mild hepatocyte vacuolation. The liver section of CCl_4 along with ethanolic extract of *E.indica* 400 mg/kg: treated group was showing mild vacuolar degeneration and mild hepatocyte swelling. The liver section of CCl_4 along with ethyl acetate extract of *E.indica* 400 mg/kg treated group has shown vacuolar degeneration, lymphocyte present in portal tract and mononuclear cell infiltration in parenchyma and portal areas. The liver section of CCl_4 along with pet ether extract of *E.indica* 400 mg/kg treated group has shown mild vacuolar degeneration and mild hepatocyte swelling.

Histopathological liver sections also revealed that the hepatic architecture was altered by hepatotoxin in Carbon tetrachloride group, whereas in the liver sections of the rat treated with the various stem bark extracts of *Erythrina indica* Lam and intoxicated with CCl_4, the hepatic architecture was not altered and was comparable with the standard drug Silymarin. The histopathological study confirms the significant hepatoprotective effect of ethanolic extract of stem bark of *Erythrina indica* Lam.

CONCLUSION

8. CONCLUSION

From the study it is concluded that

- The different extracts of the stem bark of *Erythrina indica* Lam possessed promising hepatoprotective activity against CCl_4 induced hepatic damage. The hepatoprotective activity of *Erythrina indica* is found out to be more in ethanolic extract.

- The activity could be due to the improvement in the antioxidant enzyme level and a decrease in free radical levels. The presence of phytochemicals such as flavonoids has been shown to be responsible for hepatoprotective activity.

- Further studies can be carried out in the future to elucidate the mechanism of action of the ethanolic extract of stem bark of *Erythrina indica* Lam, which may then be followed and clinical studies to establish its efficacy in humans.

REFERENCES

9. REFERENCES

1. Sembulingam k, Prema Sembulingam. Essentials of Medical Physiology. 5th ed. New Delhi: jaypee brothers Publishers;2010.P.243-247.
2. https://en.wikipedia.org/wiki/Liver physiology
3. Dineshkumar G. Rajakumar R, Mani P and Johnbastin TMM. Hepatoprotective activity of leaves extract of *Eichhornia Crassipes* against CCl_4 induced hepatotoxicity in albino rats .International Journal of Pure and Applied Zoology.2013;1(3):209-212.
4. Elamathi S, Jayshree N and Priyadharshini R. Evaluation of hepatoprotective activity of Pongamia Pinnata Linn., seed extract against carbon tetrachloride induced hepatotoxicity in Wistar rats. World Journal of Pharmacy and Pharmaceutical Sciences.2015;5(8):1542-1554.
5. http://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-point-of-view-articles-collection/global-burden-of-liver-disease-a-true-burden-on-health-sciences-and-economies
6. Di Martino S, Rainone A, Marotta G, Mazzarella N, Pugliese S and Rinaldi S. Nutraceutical agents with hepatoprotective effects in cancer patients.2016;3(4):788
7. Faheem Amir, Wan Sinn Yam and Yen Chin Koay. Phytochemical constituents and biological activities of *Erythrina indica*. European Journal of Chemistry.2011;2(4):561-565.
8. Thomas Boyer D, Michael Manns P, Arun Sanyal J. A Text book of liver disease Zakim and boyers hepatology.6th ed. Elsevier publishers;2006.P.36.

9. https://en.wikipedia.org/wiki/Liver_disease

10. Thomas Boyer D, Michael Manns P, Arun Sanyal J. A Text book of liver disease Zakim and boyers hepatology.6th ed. Elsevier publishers;2006.P.64,424-425.

11. Robbin and Cotran. Phathologic Basis of Disease. South Asia Ed. Elsevier publishers;2016.P.821-854.(Vol-II).

12. Ramadori G, Moriconi F, Malik I, and Dudas J. Physiology and Pathophysiology of Liver inflammation, damage and repair. Journal of Physiology and Pharmacology.2008;59(1):107-117.

13. Aashish pandi, Tarun sachdeva and Pallavi bafna. Drug induced hepatotoxicity. A review journal of applied pharmaceutical science.2012;2(5):233-243.

14. Mc Graw hill and Kansas.The basic science of poisions. 6th ed.

15. Nitin Dixit, Sanjula Baboota, Kanchan Kohli, Ahmad S, Javed Ali. Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. Indian journal of Pharmacology.Aug2007;39(4):172-179.

16. Chopra RN, Nayar SL. Glossary of Indian Medicinal Plants.1996 ed. Bangalore: National institute of science communication.1956.P.111.

17. Post-White J, Ladas EJ, Kelly KM. Advance in the use of milk thistle (*silybum marianum*). Integr Cancer Therapeutics.2007;6(20):104-109.

18. Sunita Shailajan, Mayuesh Joshi, Bhaves Tivari. Hepatoprotective activity of *Pamelia pelata (Huds)* Ach against CCl_4 induced liver toxicity in albino Wistar rats. Journal of Applied Phamaceutical Science.2014;4(02):070-074.

19. Nkengfack AE, Azebaze AGB, Waffo AK, Fomum ZT, Meyer M, Van Heerden FR. Cytotoxic isoflavones from *Erythrina Indica*. Phytochemistry.2000;58(7):1113-1120.

20. Rupesh Pingale and Gouri Kumar Dash. A Review on Ethnopharmacology, Phytochemistry and Bioactivity of *Erythrina Indica* Lam (Fabaceae).2014;3 (6):487-490.

21. Waffo AK, Azebaze GA, Nkengfack AE, Fomum ZT, Meyer M, Bodo B, Van Heerden FR. Indicanine B and C, two isoflavonoid derivatives from the root bark of *Erythrina Indica*.Phytochemistry.2000;53(8):981-985.

22. Mujahid M, Hussain T, Siddiqui HH , Hussain A. Evaluation of hepatoprotective potential of *Erythrina Indica* leaves against antitubercular drugs induced hepatotoxicity in experimental rats. Elsevier publishers; 2016.P.1-2.

23. Yashwant Kumar A, Nandakumar K, Handral M, Sahil Talwar, and Daniel Dhayabara. Hypoglycaemic and anti-diabetic activity of stem bark extracts *Erythrina Indica* in normal and alloxan-induced diabetic rats. Elsevier Publishers;Jan 2011.19(1):35–42.

24. Kamalraj R, Vijay Aanandhi Y. Effect of *Erythrina Indica* on stress induced alteration on lipid profile in rats. Asian Pacific Journal of Tropical Disease. 2012;S949-S951.

25. Kumar P, Adarsh Verma M1, Kavitha D, Kranthi Kumar A, Anurag KB In-vitro antioxidant and antiinflammatory activities of *Erythrina Indica* bark.Dec2010;5(3):181-184.

26. Mohd mujahid, Hefazat Husain Siddiqui. *In- vitro* evaluation of free radical scavenging activity Of *Erythrina Indica* Leaves. Journal of Drug Delivery and Therapeutics.2014;4:1.

27. Meena Agarwal, Renu Sarin. Screening of antimicrobial potential of flavonoids extracted from *Erythrina indica.* International Journal of Pharma Research & Review,Jan 2014;3(1):21-27.

28. Suthar S, Ashok P, Parsanna GS, Singh DP, Kyada A. Evaluation of analgesic activity of aqueous extract of aerial part of *Erythrina indica* in rodents. IJPTB.2014;1(1):11-16.

29. Jesupillai M, Palanivelu M, Rajamanickam V and Sathyanarayanan S. Anticonvulsant effect of *Erythrina Indica* Lam.2008;P.744-747.

30. Verma SM, Amrisha, Prakash J, Sah VK. Phyto-pharmacognostical investigation and evaluation of anti-inflammatory and sedative hypnotic activity of the leaves of *Erythrina Indica* Lam.Dec 2005.25(2):79-83.

31. Vadivel, Vellingiri, Biesalski K, Hans. Phenolic content in traditionally processed *Erythrina indica* L., seeds: antioxidant potential and Type II diabetes related functionality.2016;3:3.

32. Soniya S and Deepa R. Evaluation of bioactive compounds and antihyperlipidemic activity of *Erythrina indica* in albino Wistar rats. 2016.10(1):33-36.

33. Kamalraj R. Antihyperlipidemic studies on leaf extract of *Erythrina Indica* Lam.2011.1(3):488-490.

34. Sakat Sachin S, Juvekar Archana R. Antiulcer activity of methanol extract of *Erythrina indica* Lam leaves in experimental animals.2009.1(6):396-401.

35. Sakat SS and Juvekar AR. Comparative study of *Erythrina indica* Lam. (Febaceae) leaves extracts for antioxidant activity.2010;2(1):63–67.

36. Patil DD, Deshmukh AK and Wadhava GC. Diuretic activity of root and bark of *Erythrina indica* lam.2011.2(7):1811-1813.

37. Jesupillai M, Palanivelu M. Anthelmintic activity of leaves Of *Erythrina Indica* Lam. The Internet Journal of Alternative Medicine.2008;7:1.

38. Faheem Amir, Wan Sinn Yam, Yen Chin Koay and Kapoor LD. Phytochemical constituents and biological activities of *Erythrina indica* Ayurvedic Medicinal Plants. 2005 ed.New Delhi:CRC press;2000.P.177-178.

39. Kirthikar KR and Basu BD. Indian Medicinal Plants. New Delhi: International book distributors;2005.P.781-784.(Vol I).

40. Gamble JS. Flora of the presidency of Madras: authority of the secretary of state for India in council.2002.P.353-354.(Vol I).

41. Chopra RN, Nayar SL. Glossary of Indian Medicinal Plants. 1996 ed. Bangalore: published by National institute of science communication.1956:111.

42. Sharma R. Medicinal Plants of India An Encyclopedia. Delhi:Daya Publishing House;2003.P.99-100.

43. Nadkarni KM. Indian medicinal plants and drugs with their medicinal properties and Uses. 2006 ed.New Delhi:Srishthi book distributors;1910.P.153-154.

44. Agroforestry.net. Holualoa, Hawaii 96725 USA: The Traditional Tree Initiative. C1997-2010. [Last updated on 2010 Jan 26, last cited on 2010 Feb 5].

45. Hort.ifas.ufl.edu. University of florida: environmental horticulture.2008. [Last updated on 2010 Jan 12. last cited on 2010 Jan 27].

46. Vaidyaratnam PS. Varies, Indian medicinal plants a compendium of 500 species.2006 ed.Kotakkal:Orient Longman Pvt.ltd;2003.P.378-381.

47. Bhakuni DS, Khanna NM. Chemical examinations of the bark of *Erythrina indica* lam. J Sci Ind Res-B.1959;18(11):494-495.

48. Waffo AK, Azebaze GA, Nkengfack AE, Fomum ZT, Meyer M, Bodo B, Van Heerden FR. Indicanine B and C, Two isoflavonoid derivatives from the root bark of *Erythrina indica*.Phytochemistry.2000;53(8):981-985.

49. Nkengfack AE, Waffo AK, Azebaze GA, Fomum ZT, Meyer M, Bodo B *et al*., Indicanine A, A new 3-Phenylcoumarin from root bark of *Erythrina indica*. J Nat Prod. 2000; 63(6):855-856.

50. Pankajamani KS, Seshadri TR. Anthoxanthins. J Sci Ind Res-B.1955;8:1-93.

51. Kafaku K, Hata C. Seed oils of formason plants IX constituents of various seed oils of leguminosae. J Chem Soc Japan.1934;55:369.

52. Bhattacharyya L, Ghosh A, Sen A. A comparative study on lectins from four *Erythrina* species. Phytochemistry.1986;25(9):2117-2122.

53. Mujahid M, Hussain T, Siddiqui HH, Hussain A. Evaluation of hepatoprotective potential of *Erythrina Indica* leaves against anti-tubercular drugs induced hepatotoxicity in experimental rats.Elsevier publishers; 2016.P.1-2.

54. Nadkarni AK. Indian materia medica . 2002 ed. Bombay:Popular Prakashan, 1955.2.P.508-509.

55. Kamboj VP. A Review of Indian medicinal plants with interceptive activity.Indian J Med Res.1988;4:336-355.

56. Khan MA, Khan,T, Ahmad Z. Barks used as source of medicine in Madhya Pradesh, India. Fitoterapia.1994;65(5):444-446.

57. The ayurvedic pharmacopoeia of india. 1st ed. New Delhi:The Controller of Publications Civil Lines;2002.P.131-132.(Part-1, Volume-II).

58. Asolkar LV, Kakkar KK and Chakre OJ. Second supplement to glossary of Indian medicinal plants with active principles.1992ed.CSIR;1992.P.300-301. (Part I (A-K).

59. Jesupillai M, Palanivelu M. Anthelmintic activity of leaves of *Erythrina indica* Lam. The Internet Journal of Alternative Medicine.2008;7:1.

60. Pushpangadan, P, Atal CK. Ethno-medico-botanical investigations in Kerala. Some primitive tribals of Western Ghats and their herbal medicine.J Ethnopharmacol.1984;11(1):59-77.

61. Sachin S, Archana RJ. Antiulcer activity of methanol extract of *Erythrina indica* Lam leaves in experimental animals. 2009.1(6):396-401.

62. Nkengfack AE, Azebaze AGB, Waffo AK, Fomum ZT, Meyer M and Van Heerden FR. Cytotoxic isoflavones from *Erythrina indica*. phytochemistry.2000;58(7):1114-1120.

63. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed. Bangladesh: Asiatic Society; 2003.P.222-223.

64. Suggs RC: Marquesan Sexual Behavior,harcourt,brace + world,inc.,new york. Book, 1966.

65. Muthusamy P, Jerad Suresh A and Balamuugan G. Antiulcer activity of *Azima tetracantha* Lam a biochemical study and esearch. J. Phama and Tech.2009;2(2).

66. Lala P K. Lab manuals of pharmacognosy. 5th edition. Calcutta. CSI Publishers and Distributors.1993.

67. Kasture A V, Mahadik K R, Wadokar S G, More H N. Chromatography. pharmaceutical analysis. 15th edition.Pune.Nirali Publication.2006.

68. http://caf.iisc.ernet.in/image/cpcsea-guidelines-latest.pdf

69. Gaurav Tiwari, Ruchi Tiwari, Awani K, Rai. Cyclodextrins in delivery systems:Applications.2010;2(2):72-79.

70. OECD Guideline for Testing of Chemicals Acute oral toxicity.2001. P.1-26.

71. Surendran S, Bavani Eswaran M, Vijayakumar M, Rao Ch V. *In vitro and in vivo* hepatoprotective activity of *Cissamelos pareira* against carbon tetrachloride induced hepatic damage.2011;49:939-945.

72. Elamathi S, Jayshree N, Priyadharshini R. Evaluation of hepatoprotective activity of *pongamia pinnata* linn., seed extract against carbon tetrachloride induced hepatotoxicity in Wistar rats.2016;5(8):1542-1554.

73. Schmidt E and Schmidt FW. Enzym .Biol.Clin.1963:3:1.

74. Reitman S. and Frankel S. Amer.J .Clin.path.1957;28-56.

75. Chemie DGK. Journal of clinical chemistry and clinical Biochemistry. 1972;10,182-192.

76. Jendrassik L and Grof P. Total and unconjugated bilirubin.Biochem Z.1938; P.297, 4.

77. Carmel Punitha S and Rajasekaran M. Antioxidant mediated defense role of *Wedelia calendulacea* herbal extract against CCl4 induced toxic hepatitis. Journal of Applied Pharmaceutical Science. 2011; 01(09):111-115.

78. Christine J, Weydert, Joseph J, Cullen. Measurement of superoxide sismutase, Catalase, and glutathione peroxidase in cultured cells and tissue.2010;5(1):51-66.

79. Adejuwon Adewale Adeneye, Olufunsho Awodele, Sheriff Aboyade Aiyeola, and Adokiye Senibo Benebo. Modulatory potentials of the aqueous stem bark extract of *Mangifera indica* on carbon tetrachloride-induced hepatotoxicity in rats. Journal of trational and complementary medicine.2015;5(2):106–115.

80. Tapas AR, Sakarkar1 DM and Kakde RB. Review article flavonoids as nutraceuticals: A review. Tropical Journal of Pharmaceutical Research. September 2008;7(3):1089-1099.

81. Agrawal AD. Pharmacological activities of flavonoids: A review.2011.4(2).1394-1398.(Vol-4).

82. Schiff R, Maddrey C, Sorrell F. Schiff's Diseases of the liver. 11th ed. A John Wiley and Sons, Ltd;17-41.

83. Muthu Gounder Palanivel, Balasubramanian Rajkapoor, Raju Senthil Kumar, John Wilking Einstein, Ekambaram Prem Kumar, Mani Rupesh Kumar *et al.*, Hepatoprotective and Antioxidant Effect of Pisonia aculeata L. Against ccl4-Induced Hepatic Damage in Rats.2008.76:203–215.

84. https://en.wikipedia.org/wiki/Glutathione_peroxidase

85. https://en.wikipedia.org/wiki/Catalase.

86. Curtis JJ and Mortiz M. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. Gastroenterol.1972.62: 84–92.

ANNEXURE

INSTITUTE OF HERBAL SCIENCE
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Director
Retd, Professor, Presidency College Chennai-5

AUTHENTICATION CERTIFICATE

Based upon the Organoleptic /macroscopic /~~microscopic~~ examination of fresh /~~market~~ Bark sample, it is certified that the specimen given by C. Balachandar, Dept. of Pharmacology, Madras Medical College, Chennai-03. is identified as below:

Binomial: Erythrina Variegata L.

Family: Papilionaceae (Fabaceae)

Synonym(s): E. indica Lam.

Regional names: Tamil:- Kalyanamurungai.

Reg.No of the certificate: PAEC|2016|3302

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: Pg: 109 .1983.

Henry, A.N. *et al.* Ibid. _____ II: _____ .1987.

Ibid. _____ III: _____ .1989.

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CERTIFICATE

This to certify that Mr.**C. BALACHANDAR**, M.Pharm II year, Institute of Pharmacology, Madras Medical College, Chennai – 600003 had submitted his protocol (Part B Application) IAEC/MMC/16/2016 for the dissertation programme to the Animal Ethical Committee, Madras Medical College, Chennai-600003.

TITLE: EVALUATION OF *INVIVO* HEPATOPROTECTIVE ACTIVITY OF *Erythrina indica* Lam. STEM BARK EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

The Animal Ethical Clearance Committee experts screened his proposal No: IAEC/MMC/16/2016 and have given clearance in the meeting held on 21.11.2016 at Anatomy Demo hall III in Madras Medical College, Chennai – 600003. His study involves only Wistar rats.

ANIMAL EXPERIMENTAL LABORATORY GOVERNMENT MADRAS MEDICAL COLLEGE * Dt. 31/03/17 CHENNAI-3.

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CME - 10 CREDIT POINTS

This Certificate is awarded to Dr. / ~~Mr.~~ / ~~Mrs.~~ C. BALACHANDAR

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Organized by the Department of Epidemiology,

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This is to certify that Mr./~~Ms./Prof./Dr.~~ C. Balachander

has participated as a Delegate / Resource person in the CME on ***"Recent Advances in Research and Therapeutics of Anti Cancer Drugs"*** *held on 20th June, 2015 organized by the Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University.*

This CME offers SIX Credit Hours.

Dr. Darling Chellathai David
Professor and HOD
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Dr. K.V. Somasundaram
Dean of Faculties

INSTITUTE OF BIOCHEMISTRY

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Continuing Medical Education Programme

"NEPHELOMETRY & ITS APPLICATION IN CLINICAL DIAGNOSIS"

as Delegate / Guest Speaker / Faculty

held on 4th June, 2016 at Seminar Hall of Institute of Biochemistry, Madras Medical College, Chennai.

CME Credit points-5, Category-II, awarded by The Tamil Nadu Dr. MGR Medical University , Chennai-32.

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