Chapter – III

HEPATOPROTECTIVE AND ANTI-INFLAMMATORY ACTIVITIES

Introduction

Liver is one of the largest organs in human body comprising 2 – 3 % of total adult body weight and primarily concerned with metabolic activity of organism. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The fact that reliable live protective drugs are explicitly inadequate in allopathic medicine (Neha and Rawal, 2000) exhorted the scientists to explore herbal remedies. But it is continuously and variedly exposed to environmental toxins and sometimes free radicals generated are so high that they overpower the natural defensive system leading to hepatic damage and cause jaundice, cirrhosis and fatty liver. Production of the reactive species depletion, lipid peroxidation, plasma membrane damage, etc, culminating into severe hepatic injury.

Liver diseases are a large public health problems in the world. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drugs in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders (Chatterjee, 2000). In view of undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. The modern medicines have little to offer for
alleviation of hepatic ailments whereas most important representatives are of phytoconstituents. The traditional system of medicine, Unani system, Chinese system of medicine have a major role in the treatment of liver ailments.

In spite of tremendous advances in modern medicine, there are no effective drug available to stimulate liver functions, offer protection to the liver from damage or help to regenerate hepatic cells. In absence of reliable liver protective drugs in modern medicine, there exist challenges for pharmaceutical scientists to explore what in plants on the basis of traditional use. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. Historically plants have been used as folk medicine to treat various diseases and are rich natural sources of antioxidants. Many plants used traditionally by indigenous people to support liver function and treat diseases of the liver.

Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. In addition, serum levels of many biochemical markers like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP) and bilirubin were also elevated. In the absence of a reliable live protective drug in the modern system of medicine, a number of medicinal preciparations in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders. Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for hepatotoxicity. The plant *Casuarina equisetifolia Forst* (locally known as Jhau gachh, Hari) belongs to the family Casuarinaceae where in the extracts of leaves exhibit anticancer properties (PJS, 1967).
Bark is used as astringent and in stomach ache, diarrhoea, dysentery and nervous disorders, antiplasmodic and anti diabetic (Chevallier 1996). Leaf juice of *Cajanus cajan* (Linn.) Huth (local name Arhar) belonging to the family Papilionaceae, is useful in treating jaundice and disease of the mouth. The plant *Bixa orellana* Linn. local name is lotkan, shidhur, belongs to the family Bixaceae where in seeds are used in fever, apetising agent and stimulant. Alkaloid of the plant *Physalis minima* Linn. (Solanaceae) may have potential use for leukemia chemotherapy (Ma, 1991). Leaves and fruits are toxic, diuretic and purgative and used in gonorrhoea and spleen disorders (Yusuf et al., 1994; Chevallier, 1996). *Caesalpinia bonduc* (Linn.) Roxb. locally named as Natakaranja in Bengali belonging to Caesalpeniaceae family is used to treat fever and roasted seeds are used to treat diabetis (Chevallier, 1996). Powder of this plant is an effective in blood dysentery (JRIM, 1979). In the traditional medical practices, followed throughout the world, herbs play a major role in the management of various liver disorders. Poly herbal formulations are preferred by the traditional healer than a single herb. The phytothrapists always preferred to prescribe chemically complex and so is our food and therefore the medicines should also be chemically complex (Mills and Bone, 2000). The different plants in the herbal mixture will have different modes of action for curing the disease and in the combined form may sometimes exhibit synergistic activity (enhance activity than that of the individual herbs). Hence a particular active principle in the pharmacological activity that it has in its plants matrix, which again highlights the importance in using the plant as a whole or a mixture of plants for treating diseases (Mills and Bones, 2000). Individual and polyherbal preparations are used for the treatment of various diseases by the local medical practitioners all over India. The constituent plants in the herbal mixture or the different combinations of the same are used in
folklore remedies. In the rural areas of India, people usually use combinations of these plants to prepare medicines, for the treatment of various diseases including liver disorders, (Sairam, 1998). Some of the constituent plants of the herbal mixture namely *Glycyrrhiza glabra* Linn., (Yamamura et al., 1997), *Hemidesmus indicus* (Prabakan et al., 2000) and *Phyllanthus amarus* (Syn. *Phyllanthus niruri*) (Rajeshkumar and Kuttan, 2000) are traditionally used and scientifically proven for the treatment of liver disorders.

Recently, WHO defined traditional medicine as therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Unfortunately, it is extremely difficult to detect early warning symptoms specific to liver metabolic imbalances and a person may suffer for a longer time from liver disorder without knowing it. The incredible role in human physiology is so daunting to researchers that they visualize the simple plant remedies might have something to offer is astonishing and incredible.

India harbours about 15% out of the 20,000 medicinal plants of the world, of which 90% of them are found growing wild in different climatic conditions. The tribal and rural population of India largely depend on medicinal plants for their health care as well as for their livestock. This attracted the attention of several botanists that led to an array of reports on ethno medicine. Medicinal plants are the main sources of chemical substances with potential therapeutic effects. The use of medicinal plants for the treatment of many diseases associated with folk medicine from different parts of the world. Naturally occurring compounds from plants, fungi and microbes are still used in pharmaceutical preparations in pure
or extracted forms. A lot of compounds were characterized from plants. The research into plants with alleged folkloric use as pain and relievers and anti inflammatory agents is definitely a fruitful logical research strategy in the search for new anti inflammatory drugs.

According to the modern concept, inflammation is a healthy process resulting from some disturbance or disease. The cardinal signs of inflammation are heat, redness, swelling, pain and loss of function. Inflammation usually involves a sequence of events which can be categorized under three phases viz – acute transient phase, delayed sub acute phase and chronic proliferate phase. In the first phase, inflammation exudates developed due to enhanced vascular permeability and led to local edema. It is followed by the migration of leukocytes and phagocytes from blood to vascular tissues which is the second phase. In the third phase, tissue degradation is followed by fibrosis. Most of the anti inflammatory drugs now available are potential inhibitors of cycloxygenase (COX) pathway of erythema, edema and pain. Hence for treating inflammatory diseases anti inflammatory agents are required. These points to the utilizations of plants possessing anti inflammatory properties. Now a days the drugs used in inflammation disorders may be either having side effects. These drugs can cause gastric or intestinal ulceration that can sometimes cause secondary anaemia.

Inflammation diseases include different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, ankylosing spondylitis, polyarthritis nodosa, systemic lupus erythematosus and oilemma of medicinal world. An array of drugs are available in the market to treat these disorders but only very few are free from toxicity. Gastrointestinal problems associated with the use of anti inflammatory
drugs are still an enduring dilemma of medicinal world. It is very important that profound research with ethno botanical plants possessing anti inflammatory properties can definitely open up new vistas in inflammatory disorders. Purified natural compounds from plants can serve as template for the synthesis of new generation anti inflammatory drugs with low toxicity and higher therapeutic value.

The leaves of *Celosia argentea* L are used in the treatment of inflammations, fever and itching. The seeds are bitter, useful in blood diseases and mouth sores. They are efficacious remedy in diarrhoea (Kirtikar, 1935). Based on ethno botanical practice the plants were investigated for anti inflammatory (Patil et al., 2003), anti pyretic (Bhujbal et al., 2006), anti diabetic properties (Patel et al., 1993). Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics eg: Opioids or non narcotics eg: hydrocortisone.

More over synthetic drugs are very successful introduction of a new product approximately 3000 -4000 compounds are to be synthesized, screened and tested whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Various herbal medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases. Though relatively little knowledge about their mechanisms of action is known many herbal preparations are being prescribed widely for the treatment of inflammatory conditions. There is a need for research and developmental work in herbal medicine because
apart from the social and economic benefits, it has become a persistent aspect of present day health care in developing countries.

Inflammations is a pathological response of mammalian tissues to a variety of hostile agents including infectious organism, toxic chemical substances, physical injury, tumour growth leading to local accumulation of plasmic fluids and blood cells (Sobota et al., 2000). Although a defence mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain and aggravate many disorders. The use of non steroidal anti inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions has adverse effects, which pose a major problems in clinical use. Hence, new anti inflammatory drugs lacking such effects are being searched for as alternatives to NSAIDs. Grierson and Alfolayan (1999) showed that *Malva parviflora* Linn possessed an inhibitory effect on some fungi but was infective against some species of bacteria. The extract of the *Eclipta prostrata* L. has the ability to act as an antidote for snake venom (Melo et al., 1994; Mors et al., 1989). Recent reports showed that the saponins isolated from this plant has anti bacterial, immunosuppressant, anti – guardian and anti venom potentials (Pithyanukul et al., 2004; Sawangjarsen et al., 2005). Phytochemically, *Eclipta prostrate* is rich in wadeoctone, eclalbasaponin, B- amyrin, Stigmasterol and luteolin 7-glucoside (Asakar et al., 1992).

In spite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cell (Chattopadhyay, 2003). In absence of reliable liver- protection drugs in modern medicine, there are a number of medicinal preparations in ayurveda recommended
for the treatment of liver disorders and (Chatterjee, 2000) their usage is in
vogue since centuries and are quite often claimed to offer significant
relief. Herbal drugs are playing an important role in health care problems
world wide, and there is a resurgence of interest in herbal medicines for
the treatment of various ailments. Hepatoprotective effect of some plants
like *Spirulina maxima* (Torres Durain *et al.*, 1999). *Eclipta alba* (Saxena
and Ali, 1998) etc has been reported. Carbon tetrachloride intoxication in
rats is an experimental model widely used to study necrotic changes in
hepatic tissues.

Currently, plant derived bioactive compounds have received
considerable attention due to their therapeutic potential as anti microbial,
anti inflammatory, anti cancer and anti oxidant activities (Rathee *et al*.,
2009). Several studies have shown the antioxidant and anti inflammatory
properties of flavonoids and saponins present in various plant extracts
(Vanacker *et al*., 1995). Medicinal plants will continue to provide a source
for generating novel drug compounds, plants may become the base for the
treatment of a new medicine or they may be used as phytomedicine for
the treatment of diseases. The primary benefit of using plant derived
medicine is that they are relatively safer than synthetic alternatives,
offering profound therapeutic benefits and more affordable treatment.
Plants with anti inflammatory have been of immense ethno medicinal
uses to mankind. The search for better alternative anti inflammatory
drugs from the bounties of our vegetation is thus a worthwhile venture.
Hence the present study was aimed at investigating the hepatoprotective
activity of leaf and callus extracts of *F. trinervia* against CCl$_4$ induced
hepatotoxic in rats.
Material and methods:

Preparation of extract:-

The leaves of *F trinervia* were collected, washed thoroughly in tap water, shade dried and powdered. The powder was extracted successively with petroleum ether, methanol, chloroform and ethanol using soxhlet apparatus. The filtrate was evaporated to dryness in a shaker water bath at 42°C. The dried extracts were stored in air tight container and placed in refrigerator.

Animals:-

Wistar albino rats (150-200g) were obtained from the Ethical committee (CPCSEA MGZ – 209 dated 28-04-2010) and were housed under standard conditions (temperature 24-28°C relative humidity 60-70% and 12 hrs dark light cycles), fed commercial rat feed (Lipton India Ltd, Mumbai, India) and boiled water ad libitum.

Experimental procedure:-

Rats were divided into six groups of six each. Group A (control), group B (CCL₄ treated), group C (CCL₄ + Silymarin (25mg/kg p.o)), group D (CCL₄ + chloroform extract (150mg/kg), group E (CCL₄ + chloroform extract (300mg/kg) and group F (callus extract (300mg/kg) and is as under:
Following are the groups of animals carried out for further experiments.

**CCL₄ induced- hepatotoxicity:**

- **Group A** - Normal control (10 ml/kg distilled water, p. o)
- **Group B** - Toxicant (1:1 oliveoil + CCL₄ - 0.7mg/kg, i.p.)
- **Group C** - Standard (Silymarin 25 mg/kg, p.o)
- **Group D** - chloroform extract of *F. trinervia* Leaves (150mg/kg, twice daily, p. o)
- **Group E** - chloroform extract of *F. trinervia* Leaves (300mg/kg, twice daily, p. o)
- **Group F** - callus (300mg/kg, twice daily, p. o)

**Toxicity studies:-**

Rats of either sex were used for acute toxicity studies and it was performed by Miller and Tainter method 20 to determine lethal dose (LD 50), CMC (0.025%) was used as vehicle to suspend the extracts and the suspension was administered intraperitoneally. One tenth of the LD 50 was used as a maximum dose of extracts tested for acute toxicity. The dose was selected for evaluation of hepatoprotective activity ie 300mg/kg i.p.

For the first seven days of study Group A & B were fed with normal lab feed and water. On the seventh and eighth day animals of Group B, C, D, E & F were administered orally with a single dose of CCL₄ with olive oil mixture (1:1 olive oil + CCL₄ - 0.7mg/kg, i.p.) alternative days. Group C animals were treated with Silymarin (25mg/kg) and Group D, E & F animals were treated orally with chloroform extract of leaves of *F. trinervia* (150mg/kg & 300mg/kg) and chloroform extract of callus of *F. trinervia* (300mg/kg) respectively. On day 8, thiopentone sodium (40 mg/kg, i.p) was injected and the sleeping time was recorded
in all the animals. The same animals were then anesthetized using anesthetic ether, 1 hour after complete recovery from thiopentone sodium effect and blood collected by retro orbital puncture, serum was separated at 2500 rpm for 15 min and biochemical parameters like ALT, AST, ALP, direct bilirubin, total bilirubin, triglycerides, cholesterol, total proteins and albumin were estimated. The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with ice-cold saline, weighed and measured the wet liver volume. Small piece of liver tissue collected and preserved in 10% formalin solution for histopathological studies. Livers of some animals were homogenized with ice-chilled 10% KCl solution and centrifuged at 2000 rpm for 10 minutes and then collected the supernatant liquid for histopathological studies.

**Models to evaluate effect of drugs on liver**

1. Ascorbic acid content in urine
2. Pentobarbitone induced sleeping time
3. Bromosulphthaline clearance test

**Biochemical analysis of blood** for

a. SGPT
b. SGOT
c. Alkaline phosphatase
d. Serum bilirubin
e. Total proteins
Morphological test - Wet weight of liver/100 gm body weight

Histopathology of liver

Serum and hepatocyte enzyme

AST i.e. Aspartate Transaminase (SGOT), and ALT i.e. Alanine Transaminase (SGPT), are both sensitive markers of hepatocellular injury. When the liver cell is injured or dies, these proteins can leak through the liver cell membrane into the circulation and serum levels will rise. ALT or SGPT is a *cytosolic enzyme* primarily present in the liver. Its normal serum level is 10-35 Karmel units/ml. ALT reversibly catalyses amino group from alanine to $\alpha$-ketoglutarate.

ALT levels are very high in patients of viral hepatitis and hepatic necrosis, 10 to 200 fold higher in patients of post hepatic jaundice, intrahepatic cholestasis and below 10 fold in patients of metastatic carcinoma, cirrhosis and alcoholic hepatitis.

AST or SGOT is a *mitochondrial enzyme* released from heart, liver, skeletal muscles and kidney. Its normal serum level is 10-40 Karmel units/ml AST reversibly catalyses transfer of amino group from aspartate to $\alpha$-ketoglutarate.

AST levels are 10 to 200-fold elevated in patients with acute hepatic necrosis, viral hepatitis, CCl$_4$ and drug induced poisoning.

Alkaline phosphatase

Serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepatobiliary disease. The
mechanism of elevated ALP levels may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells. Principle involved in estimation of alkaline phosphatase:

ALP hydrolyses substrate P-nitrophenyl phosphate with the formation of P-nitrophenol and liberation of phosphate ion.

**Serum bilirubin**

Estimation of bilirubin, metabolic product of the break down of haemoglobin is one of the better liver function tests. Normally, 0.25 mg/dl of conjugated bilirubin is present in the blood of an adult. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin treatment such as Gilbert’s disease. Bilirubin in serum reacts with diazole reagent in the presence of alcohol, after the proteins had been removed by precipitation.

**Serum protein**

Liver cells synthesize albumin, fibrinogen, prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha foetoproteins and acute phase reactant proteins. The blood levels of these plasma proteins are decreased in extensive liver damage.

**Morphological parameters**

Morphological parameters like weight of the animals, weight and volume of the liver have also been used to evaluate the protective effect of the drug. Hepatotoxicity causes loss in liver weight/100 gm body weight of rats.
**Hepatoprotective activity:**

It was planned to evaluate the following parameters in all the above models.

**Physical parameters:**

Wet liver weight and wet liver volume

**Biochemical parameters:**

The following parameters were estimated.

1) Serum glutamate pyruvate transaminase (SGPT/ALT)
2) Serum glutamate oxaloacetate transaminase (SGOT/AST)
3) Serum alkaline phosphatase (SALP)
4) Serum direct bilirubin
5) Serum total bilirubin
6) Serum cholesterol
7) Serum triglycerides
8) Serum total proteins
9) Serum albumin

**Functional parameters:**

Hepatotoxins induced prolongation of thiopentone sleeping time and mortality in albino rats.

**Histopathological studies**

**A) Physical Parameters:**

**Determination of wet liver weight:**

Animals were sacrificed and livers were isolated and washed with saline and weights determined by using an electronic balance. The liver weights were expressed with respect to its body weight i.e. gm/100gm.
Determination of wet liver volume:

After recording the weight all the livers were dropped individually in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

B) Functional parameters:
On the last day, thiopentone sodium (40 mg/kg, i.p) was injected and the sleeping time was recorded in all the animals.

C) Biochemical parameters:
The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer’s instruction manual provided in the kit. (Coral clinical systems, Verna Goa, India) using Semi Auto analyser (ARTOS).

1) Estimation of Serum SGPT (UV- Kinetic method):
Principle:

SGPT catalyses the transfer of amino group from L-Alanine to 2-oxo glutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDL in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGPT activity.

\[
\text{L- alanine} + \text{2- oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L gluta} \\
\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{L- Lactate} + \text{NAD}
\]
Where:-
ALT: Alanine amino transferase
LDH: Lactate dehydrogenase

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Sample (µl)</th>
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<tbody>
<tr>
<td>Working reagent</td>
<td>1000</td>
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<tr>
<td>Sample</td>
<td>100</td>
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</table>

Mix well and aspirate

2) Estimation of serum SGOT (UV- kinetic method):

**Principle:**

SGOT catalyses the transfer of amino group from L- Aspartate to 2-oxo glutarate with the formation of oxaloacetate and L-glutamate. The rate of this reaction is monitored by an indicator reaction coupled with malate dehydrogenase (MDL) in which the oxaloacetate formed is converted to malate ion in presence of NADH. The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGOT activity.

\[
\text{L- Aspartate} + 2\text{- oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L glutamate}
\]
\[
\text{Pyruvate} + \text{NADH} \xrightarrow{\text{MDH}} \text{L- Malate} + \text{NAD}
\]
\[
\text{Sample} + \text{NADH} \xrightarrow{\text{LDH}} \text{L- Lactate} + \text{NAD}
\]

Where:-
AST: Aspartate amino transferase
MDH: Malate dehydrogenase
LDH: Lactate dehydrogenase
Mix well and aspirate.

3) Estimation of serum alkaline phosphatase (ALP):

**Principle:**

Estimation of serum alkaline phosphatase hydrolyses p-nitrophenyl phosphate in the presence of oxidizing agent Mg$^{2+}$. This reaction is measured as absorbance is proportional to the ALP activity.

$$\text{P nitro phenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALP, Mg}^{2+}} \text{p- nitrophenol} + \text{Phosphate}$$

Mix well and aspirate.

<table>
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<th>Pipette</th>
<th>Sample ($\mu$l)</th>
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<tr>
<td>Working reagent</td>
<td>1000</td>
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<td>Sample</td>
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</table>

4) Estimation of serum bilirubin:

**Principle:**

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form a pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly react in acidic medium. However, indirect and unconjugated bilirubin is solubilised using a surfactant and then it reacts similar to direct bilirubin.
Mix well. Incubate for 5 minutes at 37°C temperature for total billirubin and direct billirubin. Read absorbance at 546/630 nm against reagent blank.

5) Estimation of serum cholesterol:

Principle:

In the presence of cholesterol esterase, cholesterol esters dissociate into cholesterol and fatty acids, Cholesterol oxidase then converts the cholesterol into hydrogen peroxide and cholessterone. In the presence of peroxidase, hydrogen- peroxide reacts with 4-amino antipyrine and phenol to form a quinoneimine dye.

The estimation of cholesterol involves the following enzymatic reaction.

\[
\text{Cholesterol esters} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol + fatty acids} \\
\text{Cholesterol + O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholesterone + H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 4\text{- Amino antipyrine + phenol} \xrightarrow{\text{POD}} \text{Quinoneimine + H}_2\text{O}_2
\]

Where:

POD: peroxidase

The absorbance of quinoneimine measured spectrometrically at 505nm was proportional to cholesterol concentration in the specimen.
<table>
<thead>
<tr>
<th></th>
<th>Blank (µl)</th>
<th>Standard (µl)</th>
<th>Sample (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Distilled water</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
</tbody>
</table>

Mix well and incubate at 37°C for 10 mins. Aspirate back followed by standard and tests then measure the absorbance of the sample and standard against blank at 510/630nm.

6) Estimation of serum triglycerides (Enzymatic method):

**Principle:**

Triglycerides + H\(_2\)O  \(\rightarrow\) Glycerol + Free fatty acids  
Glycerol + ATP  \(\rightarrow\) Glycerol-3-phosphate+ ATP  
Glycerol-3- phosphate +O\(_2\)  \(\rightarrow\) DHAP +H\(_2\)O\(_2\)  
2H\(_2\)O\(_2\) + 4 AAP  \(\rightarrow\) Quinoneimine dye + 4H\(_2\)O

Where:

GK : Glycerolkinase  
GPO : Glycerol-3-phosphate oxidase  
DHAP : Dihydroxy hydrogen acetone phosphate  
ATP : Adenosine triphosphate  
AAP : Amino antipyrine  
LPL : Lipoprotein lipase  

The intensity of chromogen quinoneimine formed is proportional to the triglyceride concentration in the sample when measured at 510 nm.
Mix well, incubate at 37°C for 10 minutes. Measure absorbance of standard sample against blank within one hour.

7) Estimation of serum total proteins:

**Principle:**

The peptide bond of proteins reacts with Cu⁺² ions in alkaline solution to form a blue violet complex (Biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tarterate is added as stabilizer while iodine is used to prevent auto reduction of alkaline copper complex. The color formed is proportional to the protein concentration and is measured at 546 nm.
Some animals of livers were homogenized with ice-chilled 10% KCl solution and centrifuge at 2000 rpm for 10 minutes. Then collected the supernatant liquid and estimate the parameters like catalase, super oxidase and lipid peroxidation.

**Histopathological studies:**

**Processing of isolated liver:** The animals were sacrificed and the liver of each animal was isolated and was cut into small pieces, preserved and fixed in 10% formalin for two days. Then the liver piece was washed in running water for about 12 hours to remove the formalin and was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with three changes for 12 hours each. After paraffin infiltration the liver pieces were subjected to automatic tissue processing unit.

**Embedding in paraffin vacuum:** Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allowed to cool.

**Sectioning:** The blocks were cut using microtome to get sections of thickness of 5µ. The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in an oven at 60°C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissue to slide.

**Staining:** Eosin is an acid stain, hence it stains all the cell constituents pink which are basic in nature i.e., cytoplasm. Haematoxylin, a basic stain which stains all the acidic cell components blue i.e.: DNA in the nucleus.
Carrageenan induced paw edema:

Overnight fasted, albino rats weighing between 100 -150g were divided into four groups of five animals each. Group I served as normal and Group II served as inducer control and received normal saline, Group III received the test substances chloroform extract and Group IV received Indomethacin (10mg/kg b. w.) through oral route. After 30 min of extract, Indomethacin, saline administration, 0.1ml of 1% w/v suspension of carrageenan was injected into the sub -plantar region of the right hind paw of each rat. The paw volume is measured by using digital Plethysmometer, immediately after injection, again at 30,60,120, 180 and 240 min intervals. Percentage inhibition of edema was calculated by using following formula

\[
\% \ \text{inhibition} = \left( \frac{V_c - V_t}{V_c} \right) \times 100
\]

Where \( V_t \) = increase in paw volume in rats treated with compound and \( V_c \) = increase in paw volume in control group of rats.

Statistical analysis:

Statistical analysis was carried out using one-way analysis of variance followed by Tukey’s Multiple Comparison Test and p values implied significance (b \( p<0.01 \), and c \( p < 0.001 \)) compared with carrageenan control.

Results: CCl\(_4\) induced toxicity:

A) Physical parameters

Wet liver weight and wet liver volume:

Carbon tetra chloride treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and chloroform extract of \( F \).
*trinervia* and callus showed significant restoration of wet liver weight and wet liver volume nearer to normal.

**Table 3.1:**

**Effect of chloroform extract of *F. trinervia* on wet liver weight & wet liver volume in CCl₄ induced hepatotoxic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Wet liver weight (gm/100gm) (Mean ± SEM)</th>
<th>Liver volume (ml/100gm) (Mean ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>10ml/kg, p.o</td>
<td>2.775 ± 0.375</td>
<td>2.65 ±0.10</td>
</tr>
<tr>
<td>B</td>
<td>Toxicant Control</td>
<td>1:1 olive oil + CCl₄-0.7mg/kg, i.p.</td>
<td>5.65 ± 0.150</td>
<td>4.80 ±0.15</td>
</tr>
<tr>
<td>C</td>
<td>Standard</td>
<td>25mg/kg, p.o + CCl₄</td>
<td>3.45 ± 0.05**</td>
<td>3.20 ±0.05**</td>
</tr>
<tr>
<td>D</td>
<td>Chloroform extract</td>
<td>150mg/kg, p.o + CCl₄</td>
<td>4.25 ± 0.10*</td>
<td>3.76 ±0.015*</td>
</tr>
<tr>
<td>E</td>
<td>Chloroform extract</td>
<td>300mg/kg, p.o + CCl₄</td>
<td>3.57 ± 0.12**</td>
<td>3.30 ±0.150**</td>
</tr>
<tr>
<td>F</td>
<td>Callus</td>
<td>300mg/kg, p.o + CCl₄</td>
<td>3.0 ± 0.22**</td>
<td>2.92 ±0.18**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-Kramer’s test.

Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01.
Fig 3.1: Effect of chloroform extract of *F. trinervia*: Wet liver weights in CCl₄ induced hepatotoxic rats.

Fig 3.2: Effect of chloroform extract of *F. trinervia*: Wet liver volume levels in CCl₄ induced hepatotoxic rats.
B) Bio chemical parameters

Effect of chloroform extract of *F. trinervia*: Biochemical parameters in CCl₄ induced hepatotoxic rats.

A) **Effect of serum marker enzymes:** Rats treated with CCl₄ developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and SALP when compared to normal control. Pretreatment with silymarin, chloroform extract showed good protection against CCl₄ induced toxicity to liver. Tukey-Kramer’s test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxicant control animals.
### Table 3.2:

Effect of chloroform extract of *F. trinervia* : SGPT, SGOT, ALP levels in CCl₄ induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>SGPT levels (U/L) (Mean±SEM)</th>
<th>SGOT levels (U/L) (Mean±SEM)</th>
<th>ALP levels (mg/dl) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>10ml/kg p.o</td>
<td>26.175±0.25</td>
<td>29.05±3.5</td>
<td>30.0±0.40</td>
</tr>
<tr>
<td>B</td>
<td>Toxicant Control</td>
<td>1:1 oliveoil + CCl₄ 0.7mg/kg, i.p.</td>
<td>110±2.50</td>
<td>198.05±2.50</td>
<td>95.95±0.550</td>
</tr>
<tr>
<td>C</td>
<td>Standard</td>
<td>25mg/kg p.o + CCl₄</td>
<td>37.5±1.0***</td>
<td>51.5±1.20**</td>
<td>38.05±0.45**</td>
</tr>
<tr>
<td>D</td>
<td>Chloroform extract</td>
<td>150mg/kg p.o + CCl₄</td>
<td>87.5±2.0*</td>
<td>139.55±4.75*</td>
<td>79.2±0.50*</td>
</tr>
<tr>
<td>E</td>
<td>Chloroform extract</td>
<td>300mg/kg p.o + CCl₄</td>
<td>50.5±1.30**</td>
<td>84.5±3.20**</td>
<td>53.65±0.250**</td>
</tr>
<tr>
<td>F</td>
<td>Callus</td>
<td>300mg/kg p.o</td>
<td>45.3±1.30**</td>
<td>76.5±2.10**</td>
<td>45.25±0.20**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer’s test. Where, * represents significant at p<0.05, ** represents highly significant at p < 0.01.
Fig 3.3: Effect of chloroform extract of *F. trinervia* : SGPT, SGOT & SALP levels in CCl₄ induced hepatotoxic rats.

**Serum direct bilirubin and total bilirubin:**

Elevation of direct and total bilirubin levels after administration of CCl₄ indicate its hepatotoxicity. Pretreatment with silymarin, chloroform extract significantly reduced levels of direct and total bilirubin levels when compared to toxic control group indicating hepatoprotective effect of chloroform extract of *F. trinervia* leaves.
Table 3.3:
Effect of chloroform extract of leaves of *F. trinervia*: Direct bilirubin and total bilirubin levels in CCl$_4$ induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Direct bilirubin levels (mg/dl) (Mean ± SEM)</th>
<th>Total bilirubin levels (mg/dl) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>10ml/kg p.o</td>
<td>0.173 ±0.0120</td>
<td>0.293±0.029</td>
</tr>
<tr>
<td>B</td>
<td>Toxicant Control</td>
<td>1:1 oliveoil + CCl$_4$-0.7mg/kg, i.p.</td>
<td>1.679 ±0.099</td>
<td>1.8±0.005</td>
</tr>
<tr>
<td>C</td>
<td>Standard</td>
<td>25mg/kg, p.o + CCl$_4$</td>
<td>0.256 ±0.012***</td>
<td>0.463±0.049***</td>
</tr>
<tr>
<td>D</td>
<td>Chloroform extract</td>
<td>150mg/kg, p.o+ CCl$_4$</td>
<td>1.26 ±0.032**</td>
<td>1.33±0.059**</td>
</tr>
<tr>
<td>E</td>
<td>Chloroform Extract</td>
<td>300mg/kg, p.o+ CCl$_4$</td>
<td>0.50 ±0.040**</td>
<td>0.73±0.110**</td>
</tr>
<tr>
<td>F</td>
<td>Callus</td>
<td>300mg/kg, p.o</td>
<td>0.35 ±0.060</td>
<td>0.54±0.205***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer’s test.
Where, * represents significant at p<0.05, ** represents highly significant at p<0.01, and *** represents very significant at p<0.001.
Fig 3.4: Effect of chloroform extract of *F. trinervia*: Direct bilirubin and total bilirubin levels in CCl₄ induced hepatotoxic rats.

**Serum total protein levels:**

Ethanol treatment considerably reduced serum total protein levels. Pretreatment with Silymarin and chloroform extract of *F. trinervia* showed a significant increase in total protein levels as compared with toxicant control group.
Table 3.4:
Effect of chloroform extract of *F. trinervia*: Serum total protein, total cholesterol & triglyceride levels in CCl₄ induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Total protein levels (gm/dl) (Mean±SEM)</th>
<th>Total cholesterol levels (mg/dl) (Mean ± SEM)</th>
<th>Triglyceride levels (mg/dl) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>10ml/kg p.o</td>
<td>5.15±0.07</td>
<td>132.6±4.59</td>
<td>0.504±0.026</td>
</tr>
<tr>
<td>B</td>
<td>Toxicant Control</td>
<td>1:1 oliveoil + CCl₄- 0.7mg/kg, i.p.</td>
<td>2.34±0.25</td>
<td>321.05±10.5</td>
<td>2.32±0.079</td>
</tr>
<tr>
<td>C</td>
<td>Standard</td>
<td>25mg/kg, p.o + CCl₄</td>
<td>4.56±0.06***</td>
<td>161±3.26***</td>
<td>0.820±0.082***</td>
</tr>
<tr>
<td>D</td>
<td>Chloroform extract</td>
<td>150mg/kg, p.o+ CCl₄</td>
<td>3.52±0.58**</td>
<td>284.5±4.33**</td>
<td>2.015±0.082**</td>
</tr>
<tr>
<td>E</td>
<td>Chloroform extract</td>
<td>300mg/kg, p.o+ CCl₄</td>
<td>4.23±0.12***</td>
<td>209±5.50***</td>
<td>1.03±0.064***</td>
</tr>
<tr>
<td>F</td>
<td>Callus</td>
<td>300mg/kg, p.o</td>
<td>3.65±0.23***</td>
<td>194±3.40***</td>
<td>0.98±0.034***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-Kramer’s test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** represents very significant at p<0.001.
Serum cholesterol and Triglycerides:

From the results it was found that rats treated with CCl₄ showed a marked increase in cholesterol and triglyceride levels when compared to normal control group. In rats pre treated with silymarin and chloroform extract of *F. trinervia* the serum cholesterol and triglyceride levels had significantly decreased when compared to toxicant control group.
Fig 3.7: Effect of chloroform extract of *F. trinervia*: Serum triglyceride levels in CCl₄ induced hepatotoxic rats.

**D) Functional parameters:**

**Thiopentone induced sleeping time**

From the results, it was found that rats treated with CCl₄ have showed a marked decrease in onset of sleep and increase in duration of sleeping time when compared against normal control group. Onset of sleep had significantly increased in the rats pre treated with silymarin and chloroform extract of leaves of *F. trinervia* while the duration of sleeping time had significantly decreased when compared to toxicant group.
Table 3.5:
Effect of chloroform extract of *F. trinervia*: Onset of sleep & Duration of sleep in CCl₄ induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Onset of time (sec) (Mean ±SEM)</th>
<th>Duration of sleeping (min) (Mean ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>10ml/kg p.o</td>
<td>179.5±2.5</td>
<td>98.75±3.775</td>
</tr>
<tr>
<td>B</td>
<td>Toxicant Control 1:1 oliveoil + CCl₄- 0.7mg/kg, i.p.</td>
<td>69±10.0</td>
<td></td>
<td>235.6±7.68</td>
</tr>
<tr>
<td>C</td>
<td>Standard 25mg/kg, p.o + CCl₄</td>
<td>162.5±7.5***</td>
<td>119±2.74***</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Chloroform extract 150mg/kg, p.o+ CCl₄</td>
<td>108.5±3.5*</td>
<td>160.83±3.712**</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Chloroform extract 300mg/kg, p.o+ CCl₄</td>
<td>129±5.0**</td>
<td>125.33±3.84***</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Callus 300mg/kg, p.o</td>
<td>135±1.2***</td>
<td>109±2.3***</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey*y*-Kramer’s test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** and represents very significant at p<0.001.
Fig 3.8: Effect of chloroform extract of *F. trinervia*: Onset of sleep & duration of sleeping in CCl₄ induced hepatotoxic rats.

E) Histopathological studies of the liver in CCl₄ induced hepatotoxicity

**Normal control group:** Sections show liver parenchyma with intact architecture. Some of the central veins show congestion. Some of the hepatocytes show mild nuclear pleomorphism with prominent nucleoli. These are seen scattered mononuclear inflammatory infiltrations within all the zones.

Fig 3.9: Micro view of liver tissue of normal rat:
**CCL\textsubscript{4} treated group:** Section studied shows liver parenchyma with effaced architecture. All the zones show areas of hemorrhage, necrosis, micro vesicular steatosis, macro vesicular steatosis, degenerative hepatocytes. These are seen aggregates of mixed inflammatory infiltration within all the zones.

![Fig 3.10 : Micro view of liver tissue of CCL\textsubscript{4} induced Rat:](image)

**Silymarin + CCL\textsubscript{4} treated group:** Section studied shows liver parenchyma with focal effaced architecture. Few of the perivenular hepatocytes and focal midzonal hepatocytes show macrosteatosis and microsteatosis. Some of the central veins and sinusoids show dilatation with focal congestion. Also seen are mild stromal inflammatory infiltration comprising of lymphocytes and macrophages.
Fig 3.11: Microview of liver tissue of CCl₄ + Silymarin (25mg/kg po):

Chloroform extract (150mg/kg) + CCL₄: Section studied shows liver parenchyma with partially effaced architecture. Some of the perivenular hepatocytes and focal midzonal hepatocytes show necrosis. While some of the hepatocytes show macrosteatosis and microsteatosis. These are seen moderate mixed inflammatory infiltration comprising of neutrophils and lymphocytes.

Fig 3.12: Micro view of chloroform extract of 150mg:
**Chloroform extract (300mg/kg) + CCL₄:** Section studied shows liver parenchyma with partial effaced architecture. Few of the perivenular hepatocytes and focal midzonal hepatocytes show macrosteatosis and microsteatosis.

![Fig 3.13: Micro view of chloroform extract of 300mg:](image)

**Callus extract (300mg/kg + CCl₄):** Sections shows liver parenchyma with intact architecture.

![Fig 3.14: Micro view of callus extract (300mg/kg):](image)
Adminstration of carrageenan (1%, 0.1ml) significantly (b p < 0.01 c p < 0.001) increased the paw edema volume at regular interval as compared to normal control. Oral administration of chloroform extract in the dose of 250mg/kg significantly (b p <0.001 c p , 0.0001 ) inhibited the carrageenan induced paw edema at 30, 60, 120,180 and 240 min interval as compared to carrageenan control and it is showed in Table 6. The chloroform extract has shown 31% at four hr, where as standard drug showed 51% of inhibition as compared to the control group.

**Table – 3.6:**
Effect of chloroform extract in carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paw Volume, ml after different time interval (Time in minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carrageenan (1%, 0.1 ml)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.06±0.03</td>
</tr>
<tr>
<td><strong>Chloroform Extract (250 mg/kg p.o.)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.37±0.07</td>
</tr>
<tr>
<td><strong>Callus extract (250mg/kg.p.o)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.21±0.04</td>
</tr>
<tr>
<td><strong>Indomethacin (10 mg/kg p.o)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9 ±0.03</td>
</tr>
</tbody>
</table>
DISCUSSION

The liver cells contain abundant enzyme biomarkers which are diagnostically important. In liver injury, damage to the membranes of hepatocytes and cell organelles allows intercellular enzymes to leak into the blood that helps to predict liver damage and/or injury (Underwood et al., 2004). SGOT, SGPT and ALP are the predominant biomarker enzymes of hepatocellular necrosis, where as ALP largely represents cholestasis. The elevated serum biomarker enzymes were significantly restored nearby to normal levels in silymarin treated groups suggesting their promising hepatoprotective activity. The restoration of biomarker enzymes might be achieved through plasma membrane stabilization of hepatocytes and cholestasis.

The CCl₄ is metabolized by CYP 450 enzyme system to trichloromethyl radical (CCl₃). This in turn reacts with molecular oxygen and gets converted to trichloromethyl peroxyradical. This radical forms covalent bonds with sulfydryl group of several membrane molecules like GSH leading to their depletion and causes lipid peroxidation (Recknagel, 1983).

Physical parameters:

Wet liver weight and wet liver volume:

In case of toxic liver, wet liver weight and wet liver volumes are increased showed in Fig 3.1 & 3.2. Toxicants induced hepatotoxicity produce fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments showed in Table 3.2. In chronic alcoholics, ethanol produces hepatomegaly. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume. It is reported that liver
mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. Treatment with chloroform extract of the leaves of *F. trinervia* significantly reduced the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant hepatoprotective activity.

**Biochemical parameters:**

**Estimation of serum marker enzymes:**

Hepatotoxin gets converted into radicals in liver by the action of enzymes & these attacks the unsaturated fatty acids of membranes in presence of oxygen to give lipid peroxides consequently. The functional integrity of hepatic mitochondria is altered, leading to liver damage showed in Table 3.2.

During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations. CCl₄ administration for 3 days alternatively significantly increased all these serum enzymes showed in Fig 3.3.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increases due to leakage of this cellular enzyme into plasma by toxicants induced hepatic injury. Serum levels of SGPT can increase due to damage of the tissues producing acute hepatic necrosis, such as viral hepatitis and acute cholestasis. Alcoholic liver damage and cirrhosis also can associate with mild to moderate elevation of transaminases. In the current study treatment of rats with chloroform extract of the leaves of *F. trinervia* significantly decreased the levels of SGPT in serum which is an indication of hepatoprotective activity.
SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis & acute cholestasis. Alcoholic liver damage and cirrhosis can also associate with mild to moderate elevation of transaminases. In the current study treatment of animals with chloroform extract and callus of *F. trinervia* significantly decreased the levels of SGOT in serum which is an indicative of hepatoprotective activity.

In case of toxic liver, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells. In the current study treatment of animals with chloroform extract and callus of *F. trinervia* and part used is leaves significantly decreased the levels of ALP in serum as an indication of hepatoprotective activity.

**Direct and total serum bilirubin:**

In case of toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such a situation can occur in generalized liver cell injury showed in Fig 3.4. Certain drugs (e.g., rifampin and probenecid) interfere with the net uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert’s disease. In the current study treatment of animals with chloroform extract and callus of *F. trinervia* and part used is leaves significantly decreased the levels of bilirubin
(direct and total) in serum which is an indication of hepatoprotective activity showed in Table 3.3.

**Cholesterol and Triglycerides:**

Toxicant induces hypercholesteremia and hypertriglyceridemia, may be due to the activation of enzyme HMG CoA reductase, the rate-limiting step in cholesterol biosynthesis. The increased serum triglyceride level in ccl\(_4\) treated rats may be due to the decreased activity of lipoprotein lipase, which is involved in the uptake of triglyceride-rich lipoprotein by the extrahepatic tissues. Pretreatment with chloroform extract and callus of *F. trinervia* reduced the elevated cholesterol and triglyceride levels, suggesting that the extracts prevented ccl\(_4\) induced hyperlipidemia probably due to their hepatoprotective activity. Treatment with chloroform extract and callus significantly reduced the levels of cholesterol and triglycerides in CCl\(_4\) induced hepatotoxic animals, indicating the hepatoprotection showed in Table 3.4.

**Total protein:**

Liver toxicity decreases the total protein level in serum due to the damage to the tissues. (Fig 3.5) Since the chloroform extract and callus of *F. trinervia* show increase in total protein level in serum of animals it possesses statistically significant hepatoprotective activity.

**Functional Parameters:**

**Thiopentone induced sleeping time:**

Toxicant also alters the metabolic activity of hepatocytes, thereby inducing hepatic damage. (Fig 3.8) Barbiturates are a class of xenobiotics that are extensively metabolized in the liver. Deranged liver function leads to delay in the clearance of barbiturates, resulting in a longer
duration of hypnotic effect. In the present study, administration of thiopentone sodium to rats pre treated chronically with a toxicant resulted in an increased duration of thiopentone sleeping time. Pre-treatment with chloroform extract and callus of *F. trinervia* decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective effect (Table 3.5).

The hepatoprotective effect of chloroform extract of *F. trinervia* was confirmed by the following measures:

**Physical parameters:**

The isolated livers from the toxicant treated (CCl$_4$) animals exhibited increase in their physical parameters like wet liver weight and wet liver volume. Indeed, extract treated animals exhibited decrease in the values of above physical parameters as an indication of hepatoprotection.

**Biochemical parameters:**

In case of toxicant treated groups there will be rise in serum marker enzymes such as SGPT, SGOT, SALP, direct and total bilirubin, cholesterol and triglycerides and decrease in the level of protein. The same is observed in liver diseases in clinical practice and hence are having diagnostic importance in the assessment of liver function.

In the present study, the Chloroform extract of *F. trinervia* significantly reduced the toxicant elevated levels of above mentioned serum marker enzymes and increase in the levels of protein. Hence, at this point it is concluded that the chloroform extract possess hepatoprotective activity.
**Functional parameters:**

In the present study, administration of thiopentone sodium to rats pretreated chronically with toxicant resulted in an increased duration of thiopentone sleep time. Pre-treatment with chloroform extract of *F. trinervia* decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective effect.

**Histopathological studies:**

In toxicant treated animals there will be severe histopathological disturbances in the cytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders.

In the present study, the chloroform extract of *F trinervia* treated group animals exhibited minimal hepatic derangements and intact cytoarchitecture of the liver was maintained, indicating hepatoprotection.

Finally based on improvement in serum marker enzyme levels, physical parameters, Antioxidant parameters, functional parameters and histopathological studies, it is concluded that the chloroform extract of *F. trinervia* possesses hepatoprotective activity and thus supports the traditional application of the same under the light of modern science.

The carrageenan induced rat paw edema is a biphasic process (Vinegar *etal.*, 1969). The release of histamine or serotonin, protease, prostaglandin and lysosome (Crunkhorn and Meacock, 1971). The ethanolic stem extracts of Rubia cordifolia Linn treated rats showed 39.13% inhibition where as the Standard indomethacin produced 76.79% (Tailor Chandra Shekar *etal.*, 2010). The extracted Eugenol from Ocimum sanctum L. showed 33% inhibition where as the Standard
Paracetemol produced 22.8\% (Kirti Thakur and K. S. Pitre, 2009). The development of edema in the paw of the rat after the injection of Carrageenan is due to release of histamine, serotonin and prostaglandin. The significant activity of the chloroform extract and the standard drug observed in the present study may be due to the inhibition of mediators of inflammation such as histamine, serotonin and prostaglandin. According to statistical analysis, chloroform extract of Flaveria trinervia prevented the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity. Further studies involving the purification of the chemical constituents of the plants and the investigations in the biochemical pathway may result in the development of a potent anti–inflammatory agent with low toxicity and better therapeutic index. Inspite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, where as plants still hold their own unique place. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti–inflammatory agents.

To conclude chronic hepatic diseases stand as one of the foremost health troubles world wide, with liver cirrhosis and drug induced liver injury accounting ninth leading cause of death in western and developing countries. Therefore, treating liver diseases with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. It may be useful to the health professionals and scholars working in the field of pharmacology to develop alternative medicine to cure different kinds of liver diseases in man and animals.