SECTION – C

STUDIES ON
HEPATOPROTECTIVE
ACTIVITY
• Introduction
• Carbon tetrachloride as a hepatotoxin
• Plant materials in hepatoprotective activity
• Pharmacological screening:
  ➢ ethanolic extract of seeds of *Ricinus communis*
• Pharmacological screening:
  ➢ chloroform extract of leaves of *Ricinus communis*
• Results and discussions
HEPATOPROTECTIVE ACTIVITY

-Introduction

Uncontrolled environmental pollution, poor sanitary conditions, xenobiotics, alcohol intoxications and the indiscriminate use of potent drugs predispose the liver to a vast array of disorders. However, infection by virus still remains as the major cause of liver disease. Global estimates\(^1\) indicate that there are about 18,000 deaths every year due to liver cirrhosis caused by hepatitis. Hepatocellular carcinoma ranks among the top ten common tumors of the world, with an average of over 2, 50,000 new cases reported every year.

The liver, weighing between 1200-1500 g is the largest solid organ in the body. Essentially the liver has four quite distinct functions.

1. It supplies bile salts and bicarbonate to assist in digestion.
2. It acts as a buffer between the gut and the systemic circulation maintaining stable levels of amino acids and glucose.
3. It synthesizes a large number of specialized proteins, carbohydrates and lipids.
4. It is a major excretory pathway for the larger and more hydrophobic metabolites, foreign substances and drugs.

Liver disorders may be classified as hepatitis (Inflammation of the liver), hepatotosis (non-inflammatory disorders or degeneration of the liver parenchyma), chronic hepatitis and liver cirrhosis\(^2\). However, there is no strict hepatological delineation of these disorders, making a similar classification of hepatoprotective agents virtually impossible.
### Types of Hepatotoxic Agents

<table>
<thead>
<tr>
<th>Inorganic agents</th>
<th>Metals and metalloids: Antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic agents</td>
<td>Natural plant toxins: Albotocin, cycasin, icterogenin, indospicins, lantana, agaione, pyrrolizidines, safrole, tannic acid.</td>
</tr>
<tr>
<td></td>
<td>Mycotoxins: Aflatoxin, cyclochlorotine, ethanol, luteoskyrin, chratoxins, rubratoxins, sterignatocystins, griseofulvin, sporidesmin, tetracycline and other antibiotics.</td>
</tr>
<tr>
<td></td>
<td>Bacterial toxins: Exotoxins (<em>C. diphtheriae</em>, <em>Cl. botulinum</em>, <em>Str. hemolyticus</em>), endotoxins, ethionine.</td>
</tr>
<tr>
<td></td>
<td>Synthetic Non-Medicinal: Haloalkanes and haloolefins, nitroalkanes chloroaromatic compounds, nitroaromatic compounds, organic amines, azo compounds, phenol and derivatives, various other organic compounds.</td>
</tr>
<tr>
<td></td>
<td>Medicinal agents: Over 100 drugs used for treatment and diagnosis.</td>
</tr>
</tbody>
</table>

The agents listed in this table vary considerably in their potential for causing hepatic injury.
Table –5
Classification of hepatotoxic agents and major characteristics of each group.

<table>
<thead>
<tr>
<th>Category of agent</th>
<th>Mechanism</th>
<th>Histological lesion</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrinsic toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>Direct physico-chemical distortion and destruction of structural basic cell metabolism.</td>
<td>Necrosis (zonal) and/or steatosis.</td>
<td>CCl₄, CHCl₃, Phosphorous</td>
</tr>
<tr>
<td>Indirect cytotoxic</td>
<td>Interference with specific metabolic pathways leading to structural injury.</td>
<td>Steatosis or necrosis</td>
<td>Ethionine, Mycotoxins</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Interference with hepatic excretory pathways leading to cholestasis</td>
<td>Bile casts</td>
<td>Ictirogenin C-17 alkylated anabolic and contraceptive steroids.</td>
</tr>
<tr>
<td><strong>Host idiosyncrasy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyper sensitivity</td>
<td>Drug allergy</td>
<td>Necrosis or cholestasis</td>
<td>Sulphonamides, PAS, Halothane</td>
</tr>
<tr>
<td>Metabolic abnormality</td>
<td>Production of hepatic metabolites</td>
<td>Necrosis or cholestasis</td>
<td>Iproniazid, Isoniazid, Halothane</td>
</tr>
</tbody>
</table>
Carbon tetrachloride as a hepatotoxin

During the early part of this century, carbon tetrachloride was first found to produce hepatic injury in man and experimental animals. The intervening years have seen thousands of reports regarding adverse effects to human health due to this particular compound carbon tetrachloride. Poisoning with carbon tetrachloride has been a well-accepted and widely used model to study the pathophysiology of inflammation, liver injury or hepatic inflammation. In the course of unraveling the mechanism by which it produces fatty liver, carbon tetrachloride has served to elucidate the pathogenesis of fatty metamorphosis induced by other etiological factors. While it can lead to a damage of number of tissues it is particularly responsible for damaging to the liver and kidneys of animals / human beings. The agent is a potent hepatotoxin. Single doses lead promptly to centrizonal necrosis and steatosis. Within a few minutes, there will be an injury to the endoplasmic reticulum, which leads to functional defects of the hepatocyte and multiple biochemical manifestations of hepatic injury.

Carbon tetrachloride, because of its high lipid solubility is well distributed in the body, but produces toxic effects that are largely confined to the liver and kidneys. The toxicity is increased by agents (e.g. phenobarbitone), which induce microsomal drug metabolizing enzymes and reduced by the inhibitors of microsomal enzymes. The microsomal mixed function oxidase system withdraws an electron from CCl₄ leaving the reactive trichloromethyl radical CCl₃•. This free radical has life time of only about 100 microseconds and
so has time to diffuse for only a short distance within the liver cell before undergoing secondary reactions. The secondary reactions, which are responsible for the biochemical damage may be of various kinds:

a) Oxidation of thiols to disulphide bonds.

b) Saturation of double bonds in lipids, proteins or nucleotides, resulting covalent attachment of free radical group of those sites.

c) Lipid peroxidation reaction in which polyunsaturated membrane lipids are converted to peroxide derivative and eventually to aldehydes and other products leading to a further cascade of reaction, which results in irreversible membrane damage.

Prolonged administration of carbon tetrachloride can lead to cirrhosis¹³ and hepatic carcinoma¹⁴. Most of the acute and chronic hepatic injuries appear to result from the action of metabolite of the toxin⁴. Chemically carbon tetrachloride is a simple, strongly non-polar molecule⁶, which undergoes metabolism in the smooth endoplasmic reticulum. A chemical characteristic that is the relative low energy for C-Cl bond which may be highly responsible to brand carbon tetrachloride as a potential hepatotoxin has been emphasized by Recknagel and Glende⁴ and Slater¹⁵. The bond association energy is progressively higher in less toxic haloalkanes and tends to be lower in more toxic haloalkanes⁴,¹¹,¹⁵.

- **Plant materials in hepatoprotective activity**

  Despite tremendous advances in modern medicine, there is hardly any drug that stimulate liver function, offer protection to the liver from damage or help the regeneration of the parenchyma cells¹⁶. While corticosteroids are
immunosuppressive agents, the side effects of which are alarming, are the only
drugs of choice in modern medicine for the management of liver ailments. Plants
and natural products are proving to be good hepatoprotectants. This is evident
from the voluminous work published on such materials and their hepatoprotective
activity\textsuperscript{17}. The importance of plant products in modern medicine even in a highly
advanced society as that of USA can be seen from the data of natural surveys\textsuperscript{18},
where in it was found that 25\% of all the prescriptions dispensed contained crude
plant materials or crude plant extracts. About 170 phytoconstituents isolated from
around 100 plants belonging to 55 families have been reported to possess liver
protective activity and about 600 commercial herbal formulations with claimed
hepatoprotective activity are being sold world wide. Of these about forty patent
poly herbal formulations, representing various Indian herbs are available in the
Indian market. For centuries, indigenous drugs, either alone or in combination
were have advocated in the traditional systems of medicine especially Ayurveda
for the treatment of liver disorders.

It was the isolation of silymarin\textsuperscript{19-23}, a flavanolignan from \textit{Silybum}
\textit{marianum} an widespread research on hepatoprotective agents all over the world.
Other important antihepatotoxic drug discoveries from plant sources include
cynarin from \textit{Cynara scolymus}\textsuperscript{24}. The discovery of diverse chemical compounds
from the natural products and synthetic compounds used in protective liver
therapy such as phospholipids, sugar alcohols, pyrimidine, purine derivatives,
vitamins, cysteine, glutathione, corticoids, androgens, penicillamine, ricinin etc.,
does not confine the activity to any particular class of compounds\textsuperscript{25}, but
emphasizes once again the complexity of liver disorders in addition to the different action, mechanisms of different pharmaceutical preparations. However, it may be noted that many of the anti hepatotoxic compounds mentioned in literature are phenolic and phenol propane derivatives. Systematic pharmacological studies are therefore well conceived and justified especially in the lignans, neolignans, higher condensed flavonoids and cinnamic acid derivatives.

It may be noted that though hepatoprotective activity was reported for various classes of phytoconstituents such as flavonoids, triterpenes, steroids, lignans, polyphenols, glycosides, saponins, volatile oils, coumarins etc., the reports on hepatoprotective activity of triterpenes, flavonoids, steroids and lignans were comparatively more.26

Extensive work was carried out on the antihepatotoxic activity of flavonoids as a class of compounds. The hepatoprotective activity of flavonoids like quercetin, luteolin, apigenin, quercitrin etc is well documented in literature27, 28. Though their mechanism of action has not been clearly understood, certain flavonoids like silybin, cyanidanol-3, quercetin and taxifolin are believed to act as antioxidants and therefore may be useful in the treatment of liver disorders, where lipid peroxidation is an important process29, 30.
PHARMACOLOGICAL SCREENING-
HEPATOPROTECTIVE ACTIVITY

- **Effect of the ethanolic extract of the seeds of Ricinus communis**
  - **Preparation of extract**
    
    Fresh seeds of the plant (2 kg) were collected, washed and Sun dried. They were then crushed to a fine powder. The dried powder (600 g) was exhaustively extracted with ethanol in batches of 200 g each in the soxhlet extractor (40 cycles for each batch). The ethanolic extract was collected. The ethanol was distilled off and the concentrate was evaporated on a water bath to get a sticky mass (10 g). This residue was used for screening various pharmacological activities.
  - **Selection of animals**

    Male Wistar albino rats weighing between 200-250 g were obtained from M/s Maharvir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed in polypropylene cages in an adequately ventilated room. (At a temperature of 25±2° with an alternating 12 hr light – dark cycle and relative humidity of 50 ±15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the regulatory body of the government (Regd.No.1048/a/07/CPCSEA). The rats were allowed to have standard rat feed pellets supplied by Hindustan Lever Co., Bombay and water *ad libitum* throughout the course of the study.
- **Preparation of Tween 80 (1%)**

  Stock suspension of Tween 80 was prepared by triturating 1gm of Tween 80 in 100ml of distilled water, was used for suspending the test compound and standard drug.

- **Acute Toxicity Study**

  Animals weighing between 200-250 g were used for the study. These were divided into 6 groups of 6 animals each. The test extract was administered orally as a suspension in Tween 80 (3 ml of 1% solution) to the different groups in increasing dose levels of 10, 40, 100, 400, 1000 and 4000mg/kg body weight.

  The animals were then observed continuously for 3 hours for general behavior, neurological and autonomic profiles for every 30 minutes for next 3 hours and finally till death after 24 hours\(^3\).

- **Hepatotoxin**

  A dose of CCl\(_4\) (0.4 ml /kg body weight, i.p.) was used as hepatotoxin to induce liver damage.

- **Standard drug**

  A dose of silymarin (1.25 mg per/kg body weight, p.o) was used as a standard drug as hepatoprotective agent. It was obtained as a gift sample from M/S Himalaya Drug Company, Bangalore.

- **Selection of Dose**

  From preliminary toxicity studies it was observed that the animals were safe for maximum dose of 4000 mg/kg body weight. But there were a few changes in the behavioral responses like alertness, touch response and
restlessness. Therefore 1/10th of maximum tolerated dose i.e. 400 mg /kg body weight was chosen for the study.

- **Hepatoprotective activity**

  Modified method of Chandra et al\(^3\)\(^2\), was adopted in this study. 24 albino rats weighing 200–250 g were used for the evaluation of the hepatoprotective activity of the ethanolic extract of the seeds of *Ricinus Communis*. These were divided into 4 groups of 6 animals each.

**Group I**

The animals served as normal control. These were orally dosed with Tween 80 (3 ml of 1% solution) daily for seven days. Blood samples were collected by direct heart puncture on 8\(^{th}\) day to estimate serum enzyme levels of SGOT, SGPT and alkaline phosphatase. In the process animals will die as a consequence due to over dose of pentobarbitone. Liver was excised off and kept in formalin for histopathological studies.

**Group II**

The animals served as CCl\(_4\) control. They were injected intraperitoneally with CCl\(_4\) (0.4ml/kg body weight) followed after 10 minutes by the oral administration of Tween 80 (3 ml of 1% solution) daily for seven days. Blood samples were collected on 8\(^{th}\) day to estimate serum enzyme levels of SGOT, SGPT and alkaline phosphatase. In the process animals will die as a consequence due to over dose of pentobarbitone. Liver was excised off and kept in formalin for histopathological studies.
Group III

The animals were treated with CCl₄ (0.4 ml/kg body weight, i.p.) for seven days. Then from the 8th day to 14th day silymarin (1.25 mg/kg body weight, p.o) was administered as a suspension in Tween 80 (3 ml of 1% solution). Blood samples were withdrawn on the 15th day by direct heart puncture and their enzyme levels of SGOT, SGPT and alkaline phosphatase were estimated. In the process animals will die as a consequence due to over dose of pentobarbitone. Liver was excised off and kept in formalin for histopathological studies.

Group IV

The animals were treated with CCl₄ (0.4 ml/kg body weight i.p.) for seven days and ethanolic extract of the seeds of Ricinus communis (0.4 g/kg body weight, orally) as a suspension in Tween 80 (3ml of 1% solution) was administered from 8th day to 14th day. Blood samples were withdrawn on the 15th day by direct heart puncture. The enzyme levels of SGOT, SGPT and alkaline phosphatase were estimated. The animals were sacrificed by pentobarbitone overdose. Liver was excised off and kept in formalin for histopathological studies.

- **Biochemical studies**

The blood samples were collected from the animals of all 4 groups by direct cardiac puncture of the anaesthetized animals. The serum was separated by centrifugation and kept at-15°C; using an autoanalyzer, the following biochemical parameters were studied.
Pharmacology

a) Serum Glutamic Oxaloacetic Transaminase (SGOT)

b) Serum Glutamic Pyruvic Transaminase (SGPT)

c) Alkaline Phosphatase (ALP)

➢ **Histopathological studies**

The liver of animals of all the four groups were taken and liver sections were performed by using microtome. The sections were then stained with haematoxylin and eosin and were observed for degeneration and necrotic changes which were graded as follows:

a) Degeneration:

   O : No degeneration

   + : Few vacuolated cells per lesion

   ++ : More than 10 cells per lesion

   +++ : More than 2 rows of vacuolated cells around necrotic zone per lesion.

b) Necrosis:

   O : No necrosis

   + : Focal necrosis of one or two cells per lesion.

   ++ : Focal necrosis of more than two cells per lesion.

   +++ : Massive centrilobular necrosis.

➢ **Statistical analysis**

Unpaired student’s t test was used to analyse the difference in biochemical parameters between control groups and treated groups.
RESULTS AND DISCUSSION

Hepatoprotective activity

Group I

Biochemical studies

The serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels stood at 44.43±1.02 U/L, and 45.02±1.1 U/L respectively. While the alkaline phosphatase level was 412.1±1.09 U/L (Table-6).

Histopathological studies

The section of liver which was exposed to Tween 80 showed normal structure of the hepatic cells (Fig-2a).

Group II

Biochemical studies

The animals of this group displayed a significant increase in SGOT (157.5 ± 1.43 U/L), SGPT (145 ± 2.1 U/L) and alkaline phosphatase (472.66 ±2.63 U/L) respectively, in comparison to those of the normal animals of group I (Table-6).

Histopathological studies

The section of liver which was exposed to CCl₄ showed centrilobular fatty degeneration, cloudy swelling and necrosis of hepatic cells (Fig-2b).
Figure 1a: Normal Control rat - Section of liver show normal hepatic cells.

Figure 1b: CCl$_4$ treated rat - Section of liver showing centrilobular fatty degeneration, cloudy swelling and necrosis of hepatic cells.
Group III

**Biochemical studies**

When compared to group II, this group exhibited a significant fall in SGOT (64.3±0.932 U/L), SGPT (62.0±0.57 U/L) and alkaline phosphatase (423.5±1.04 U/L) (Table-6).

**Histopathological studies**

The section of which was exposed to CCl₄ and Silymarin in succession showed normalcy of hepatic cells, central vein and portal vein (Fig-2c).

Group IV

**Biochemical studies**

The SGOT (83.5±1.32 U/L), SGPT (86.3± 1.06 U/L) and alkaline phosphatase (426.5±2.30 U/L) decreased significantly when compared to the CCl₄ treated animals of group II (Table-6).

**Histopathological studies**

The section of liver which was exposed to CCl₄ and treated with ethanolic extract of the seeds of *Ricinus communis* obtained by the author in succession revealed normalcy of hepatic cells (Fig -2d).
Fig 2c: Section of the rat liver treated with CCl$_4$ followed by silymarin.

Fig 2d: Section of the rat liver treated with CCl$_4$ followed by ethanolic extract of the seeds of *Ricinus communis*. 
Table - 6

Effect of ethanolic extract of the seeds of *Ricinus communis*
on CCl₄ induced biochemical changes in male albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal)</td>
<td>44.43±1.02</td>
<td>45.02±1.1</td>
<td>412.1±1.09</td>
</tr>
<tr>
<td>Group II (CCl₄)</td>
<td>157.5±1.43*</td>
<td>145±2.1*</td>
<td>472.66±2.63*</td>
</tr>
<tr>
<td>Group III (CCl₄ +Silymarin)</td>
<td>64.3±0.932*</td>
<td>62.0±0.57*</td>
<td>423.5±1.04*</td>
</tr>
<tr>
<td>Group IV (CCl₄ +Ethanolic extract)</td>
<td>83.5±1.32*</td>
<td>83.6±1.06*</td>
<td>426.5±2.30*</td>
</tr>
</tbody>
</table>

*P < 0.001 compared to control

Values are mean ±SEM, of six animals in each group.

Data was analysed by unpaired ‘t’ test.
Fig 3: Variation in enzyme upper levels for all the treated groups

![Graph showing enzyme upper levels for different groups]

Fig 4: Variation in enzyme lower levels for all the treated groups

![Graph showing enzyme lower levels for different groups]
Histopathological studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Degeneration</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CCl₄</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CCl₄ + Silymarin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CCl₄ + Ethanolic extract</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No degeneration as well as no indication of necrosis was observed for the section of the liver treated with Tween 80. The section of liver treated with carbon tetrachloride showed more than two rows of vacuolated cells around necrotic zone per lesion and massive centrilobular necrosis. In case of the section of liver treated with carbon tetrachloride and sylimarin in succession exhibited a few vacuolated cells per lesion and focal necrosis of one or two cells per lesion. The section of liver treated with carbon tetrachloride and ethanolic extract of the seeds of *Ricinus communis* obtained by the author in succession does not revealed any degeneration and as well as necrotic changes, which indicates that the ethanolic extract of the seeds of *Ricinus communis* has shown fruitful results in the hepatotoxic protective activities.
Effect of the chloroform extract of the leaves of *Ricinus communis*

Modified Chandra et al\(^{25}\) method was adopted for this study. The chloroform extract obtained as per the procedure described in chemical investigation was taken up for this study. The processes of evaluation of hepatoprotective activities of seeds of *Ricinus communis* was carried out with details of the activities like - acute toxicity study, hepatotoxin, selection of dose, hepatoprotective activity, biochemical and histopathological studies. Based on the above studies results and discussions pertaining to the observations were narrated here under.
RESULTS AND DISCUSSION

Hepatoprotective activity

Group I

Biochemical studies

The Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) values were found to be 89.88±1.25 U/L and 35.16 ± 0.92 U/L respectively whilst the alkaline phosphatase value was found to be 100.05±1.125 U/L (Table-7).

Histopathological studies

The liver exposed to Tween 80 revealed the following facts: The black spots appearing at the central portion represent glycogen whereas white spots indicate the presence of vacuoles. A prominent blood vessel with normal appearance having fewer vacuoles can also be witnessed from (Fig-5a).

Group II

Biochemical studies

The animals of this group displace significant increase in values of SGOT (141.4 ± 17.286 U/L), SGPT (77.5±1.79 U/L) and alkaline phosphatase (379.98 ±48.786 U/L) respectively in comparison to those of the normal animals of Group I (Table-7).

Histopathological studies

The liver exposed to CCl₄ revealed the following facts: The cells were ruptured, vacuoles are clearly visible covering large area showing necrosis of liver cells. Number and size of vacuoles are much more than normal. The walls of blood vein, bile ductioles are in the form of waves and broken at several places. The epidermis is also swollen and wavy which shows the swelling and oedema in the liver tissues. Several cells are not nucleated and cytoplasm of a few cells is dissolved (Fig-5b).
Fig 5a: Section of the rat liver (normal control).

Fig 5b: Section of the rat liver treated with $\text{CCI}_4$.
Group III

Biochemical studies

When compared to group II, this group exhibited a significant fall in the values of SGOT (119.58±4.86 U/L), SGPT (53.0±1.450 U/L) and alkaline phosphatase (290.98±1.26 U/L) (Table-7).

Histopathological studies

Liver exposed to CCl₄ and treated with silymarin in succession revealed normal hepatocytes and mild inflammation (Fig-5c).

Group IV

Biochemical studies

The values of SGOT (126.4±1.34 U/L), SGPT (60.83±0.976 U/L) and alkaline phosphatase (304.88 ±2.708 U/L) decreased significantly when compared to the CCl₄ treated animals of group II (Table-7).

Histopathological studies

In the case of liver exposed to CCl₄ and treated with chloroform extract of the leaves of *Ricinus communis* obtained by the author in succession led to the following observations: vacuoles or necrosis visible but the size and number of vacuoles are less than that of controls. The walls of blood vessel are slightly damaged (Fig-5d).
Fig 5c: Section of the rat liver treated with CCl₄ followed by silymarin.

Fig 5d: Section of the rat liver treated with CCl₄ followed by the chloroform extract of the leaves of *Ricinus communis*. 
Table –7

Effect of chloroform extract of the leaves of *Ricinus communis*
on CCl₄ induced biochemical changes in male albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal)</td>
<td>89.88±1.256</td>
<td>35.16±0.92</td>
<td>100.05±1.25</td>
</tr>
<tr>
<td>Group II (CCl₄)</td>
<td>141.4±17.286*</td>
<td>77.5±1.79*</td>
<td>379.98±48.786*</td>
</tr>
<tr>
<td>Group III (CCl₄ +Silymarin)</td>
<td>119.58±4.86*</td>
<td>53.01±1.45*</td>
<td>290.98±1.26*</td>
</tr>
<tr>
<td>Group IV (CCl₄ +Chloroform extract)</td>
<td>126.4±1.34*</td>
<td>60.83±0.976*</td>
<td>304.88±2.708*</td>
</tr>
</tbody>
</table>

*P < 0.001 compared to control

Values are mean ±SEM, of six animals in each group.

Data was analysed by unpaired ‘t’ test.
Fig 6: Variation in enzyme upper levels for all the treated groups

Fig 7: Variation in enzyme lower levels for all the treated groups
Histopathological studies

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<tr>
<td>Normal</td>
<td>0</td>
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</tr>
<tr>
<td>CCl₄</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CCl₄ + Silymarin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CCl₄ + Chloroform extract</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

No degeneration as well as no indication of necrosis was observed for the section of liver treated with Tween 80. The section of liver treated with carbon tetrachloride showed more than two rows of vacuolated cells around necrotic zone per lesion and massive centrilobular necrosis. In case of the section of liver treated with carbon tetrachloride and sylimarin in succession exhibited a few vacuolated cells per lesion and focal necrosis of one or two cells per lesion. However the section of liver treated with carbon tetrachloride and chloroform extract of the leaves of *Ricinus communis* obtained by the author in succession revealed a few vacuolated cells per lesion and focal necrosis of one or two cells per lesion. The above findings indicate that the ethanolic extract of the seeds of *Ricinus communis* is effective in the hepatoprotective activity.