

CHAPTER 6

HEPATOPROTECTIVE ACTIVITY

6.1 INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [74]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But when it is continuously and variedly exposed to environmental toxins, chemicals like CCl_4 , drug habits, alcohol, infections and autoimmune disorders, prescribed (antibiotics, chemotherapeutic agents) cum over-the-counter drugs can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [75]

Liver damage

The main causes of liver damage are

- Chemical agents, certain antibiotics, peroxidised oil, aflatoxins, CCl_4 , chlorinated hydrocarbon etc.
- Excess consumption of alcohol.

- Infection and autoimmune disorders.

Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid per oxidation and other oxidative damages in liver. Enhanced lipid per oxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis [76].

Thus liver diseases are one of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders [77].

Herbal drugs provide significant source of hepatoprotective drugs. Mono and poly-herbal preparations have been used in various liver disorders. According to an estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use as hepatoprotective [78].

Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Several authors have reported favourable results with herbal drugs (mostly in form of extracts) either in animal or in human studies. *Ginkgo biloba* L., *Echinacea purpurea* L., *Hypericum perforatum* L. and *Cimicifuga racemosa* (L.) Nutt. were successfully subjected to clinical trials after preclinical evaluation.

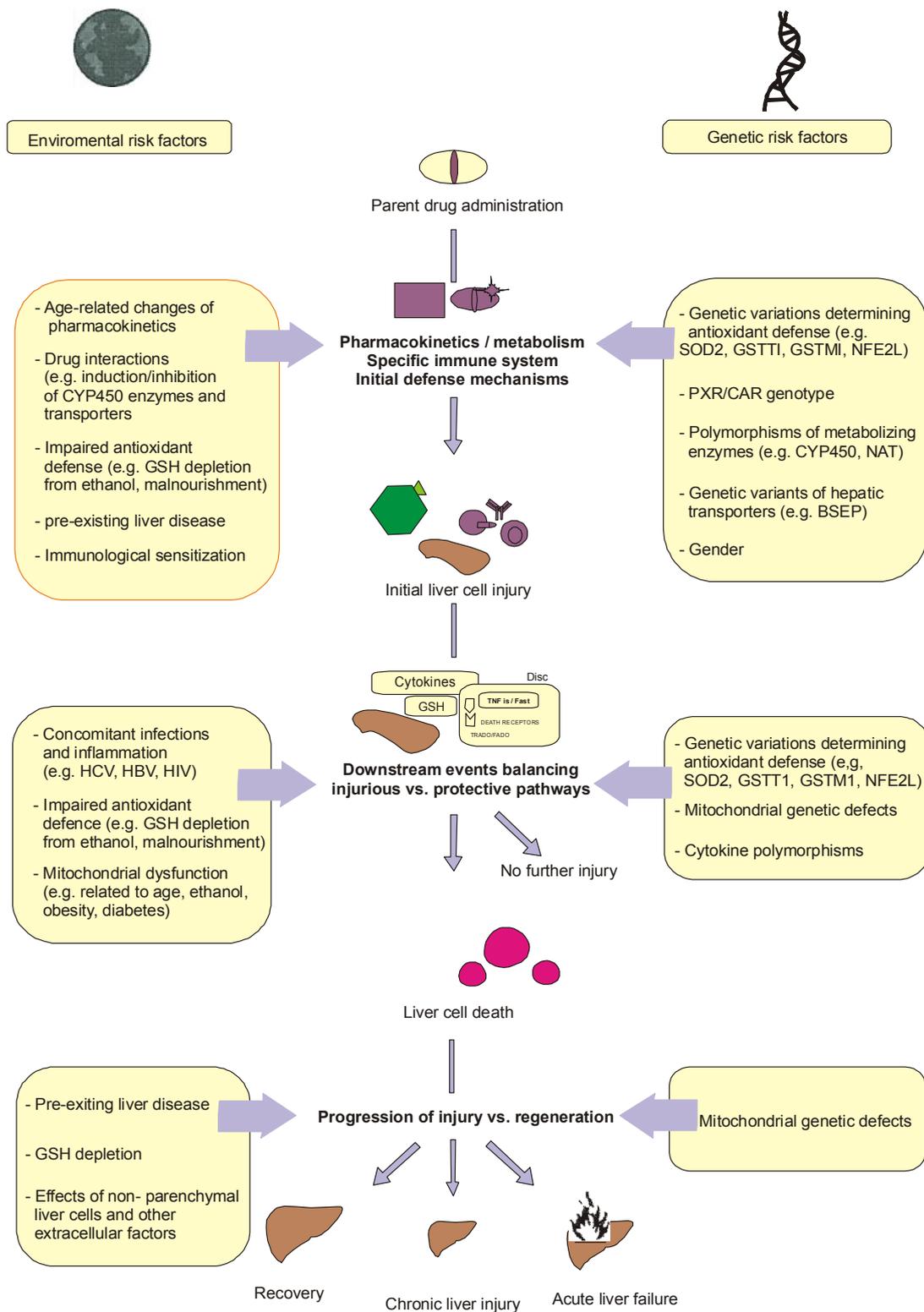


Figure 6.1 Risk factors for hepatotoxicity

Silybum marianum L., the reputed hepatoprotective, has remained a golden standard in the treated of liver ailments. In India, a study has reported that *Picrorrhiza kurroa* Royle is more potent than *Silybum marianum* as hepatoprotective agent (however, this study is not complete in all aspects).

Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, Penicillamine and corticosteroids are inconsistent at best and the incidence of side-effects profound. Too often the treatment is worse than the disease. Physicians and patients are in need of effective therapeutic agents with a low incidence of side-effects. Plants potentially constitute such a group. Several hundred plants have been examined for use in a wide variety of liver disorders. The latter category of plants include: *Silybum marianum* (Milk thistle), *Picrorrhiza kurroa* (Kutkin), *Curcuma longa* (Turmeric), *Camellia sinensis* (Green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (Liquorice), *Allium sativa* (Garlic), *Andrographis paniculata*, *Phyllanthus niruri* and *Eclipta alba* are proven hepatoprotective medicinal herbs, which have shown genuine utility in liver disorders. These plants are used widely in hepatoprotective preparations and extensive studies have been done on them. There are number of phytoconstituents from plants which have exhibited hepatoprotective activity. Recent progress in the study of such plants has resulted in the isolation of about 170 different phytoconstituents from plants belonging to about 55 families, which exhibit hepatoprotective activity [79].

6.1.2 Herbal Formulations used in Liver Disorder

- a) LIV-52: It is a non-toxic hepatoprotective substance from The Himalaya Drug Co. Liv.52 can improve the subjective condition and clinical parameters in patients with liver damage, in particular in alcoholic liver damage.

- b) LIMARIN (Capsules and Suspension): It has a potent hepatoprotective and free radical scavenging (antioxidant) action. LIMARIN® is developed from the active extract of the fruit of *Silybum marianum*, or the milk thistle. It is basically a European herbal product.
- c) CIRRHITIN: Cirrhitin is a natural medicine formulated specifically to treat Cirrhosis of the liver and marketed by CCNOW.
- d) SRILIV (Capsules): Hepatoprotective, Herbal Capsules for Loss of Appetite, Alcoholic Liver Damage, Hepatitis and Jaundice.

Some other polyherbal preparations such as Livex, HD-03, Hepatomed, Live 100 and Hepatoguard with proven efficacy are also used in different types of liver disorders [80].

6.1.3 Investigation of Liver Function

The liver function tests are employed for accurate diagnosis, to assess the severity of the damage, to judge the prognosis and to evaluate the therapy. The routinely performed liver function tests (LFT) are as follows:

Hepatoprotective activity can be most easily evaluated / screened with the aid of several model systems of liver damage in experimental animals. In all test model systems conditions for liver damage are implemented and an attempt is made to counteract this toxicity with the substance / preparation under test. The magnitude of the protective effect can be measured by estimating the enzymes and the rate of survival and can be verified histologically.

Experimental Models for Hepatoprotective Screening

Chemical reagents and drugs which induce liposis, necrosis, cirrhosis, carcinogenesis and hepatobiliary dysfunctions in experimental animals are classified as hepatotoxins. The most important ones used are carbon tetrachloride (CCl₄), thioacetamide (TAA), D-galactosamine, chloroform, ethyl alcohol and Pyridine.

6.2 MATERIALS AND METHODS

6.2.1 Requirements

Carbon tetrachloride, Diethyl ether, Formaldehyde Solution, Glutathione Reduced (GSH), were procured from SB Fine – chemicals 5,5” – Dithio-bis-2initro benzoic acid, Sodium hydrogen phosphate monobasic, Sodium hydrogen phosphate dibasic, 1,1,3,3 tetramethoxypropane, were procured from Sigma Chemical Company, Ethylenediamine tetra acetic acid (EDTA) from Qualigens fine Chemicals, Hydrogen Peroxide Solution from Merck Olive Oil from Sasso, Italia. Silymarin, standard drug was procured from Sigma Chemical Company, USA. Thiobarbituric acid, from HI media, Trichloro acetic acid, from Ranbaxy fine chemicals, 50% Ethanol was procured from Qualigens fine chemicals. All the reagents used were of analytical grade.

For the estimation of hepatic biochemical markers like Aspartate Serum Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) [81], Total Bilirubin (TB) [82] and Total protein (TP), Triglycerides (TG) Gammaglutamyl transferase (GGT) levels [83] using standard kits. The enzyme levels were estimated using commercial kits from Erba Diagnostics, Germany.

The following experimental rat model is employed in the present study for inducing acute hepatic damage by CCl₄ [84].

The animals were divided into five groups of six rats each. A suspension of carbon tetrachloride in olive oil (1:1)

Group I : The animals of this group served as normal control received olive oil for 10 days.

Group II : The animals received a mixture of olive oil 1 ml/kg and carbon tetrachloride 1 ml/kg (1:1) intraperitoneal for the last three days [88].

Group III : The animals were injected intraperitoneal standard Silymarin (100mg/kg) and a mixture of carbon tetrachloride 1 ml/Kg and olive oil 1 ml/Kg.

Group IV : The animals were injected intraperitoneal with a mixture of carbon tetrachloride 1ml/kg and olive oil 1ml/kg (1:1) prior to the administration of 200 mg / kg body weight of MELA for ten days.

Group V : The animals were injected intraperitoneally with a mixture of carbon tetrachloride 1ml/kg and olive oil 1 ml/kg (1:1) prior to the administration of 400 mg / kg body weight of MELA for ten days.

The rats were kept overnight fasting after 10days and blood samples were collected by retro orbital puncture under ether anaesthesia and the serum was used for the estimation of hepatic biochemical markers like AST, ALT,ALP, TB,TP, TG and GGT levels using standard kits. The enzyme levels were estimated and the results were expressed as U/l.

6.2.2 Determination of Biochemical Parameters

a) Determination of Serum Bilirubin

Principle

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to Bilirubin concentration. Direct Bilirubin, being water soluble directly reacts in acidic medium. However indirect or in conjugated Bilirubin is solubilised using a surfactant and then it reacts similar to Direct Bilirubin.

Procedure

Total bilirubin / Direct bilirubin

In different tubes, Pipetted 500 μ l of working reagent into marked test tubes as Blank, Standard and Test and to this add 25 μ l distilled water, calibrator and Serum respectively.

Mix well, incubated for 5 minutes at 37 ° C for Total Bilirubin and Direct Bilirubin. Read absorbance at 546/630 nm against reagent blank using semi auto analyzer by endpoint method. The units are expressed as mg/dl. was calculated by the following formula (6.1, 6.2)

$$\text{Total Bilirubin} = \text{Absorbance of Test} \times 23 \text{ (Factor)} \quad (6.1)$$

$$\text{Direct Bilirubin} = \text{Absorbance of Test} \times 17 \text{ (Factor)} \quad (6.2)$$

b) Determination of Aspartate alanine Transaminase (ALT)

Procedure

In different tubes Pipetted 1000 μ l of working reagent into marked test tubes as Blank and Test and to this added 100 μ l working reagent and Serum respectively. Mix well, incubated for 5 minutes at 37 °C and read the

absorbance at 340 nm. A decrease in the absorbance against Reagent blank was measured for 180 seconds at an interval of 30 seconds using semi auto analyzer by kinetic method. The Units were expressed as IU/L and the result was calculated by the following formula (6.3)

$$\text{ALT activity [IU/L]} = \Delta A / \text{min.} \times \text{Factor (1768)} \quad (6.3)$$

c) Determination of Aspartate amino Transferase (AST)

Procedure

In different marked test tubes as Blank and Test, Pipetted 100 μ l working reagent in both the tubes and 10 μ l Serum to the tube marked as test. Mixed well, incubated for 5 minutes at 37 °C and read the decrease in absorbance at 340 nm against Reagent blank for 180 seconds at an interval of 30 seconds using semi auto analyzer by kinetic method. The Units are expressed as IU/L. The result was calculated by the following formula (6.4)

$$\text{AST activity [IU/L]} = \Delta \text{Absorbance} / \text{min.} \times \text{Factor (1768)} \quad (6.4)$$

d) Determination of Gamma – Glutamyl Transferase (GGT)

Procedure

In different tubes marked as Blank and Test added 1000 μ l working reagent and 100 μ l Serum respectively. Mixed well and read the increase in the absorbance against Reagent blank at 405 nm for 180 seconds at an interval of 30 seconds using semi auto analyzer by kinetic method. The Units are expressed as IU/L. The $\Delta A / \text{min}$ was calculated by the following formula (6.5)

$$\text{Activity of GGT at 37 } ^\circ\text{C (IU/L)} = (\Delta \text{Absorbance} / \text{min}) \times \text{Factor (1158)} \quad (6.5)$$

6.2.3 Antioxidant Study

Preparation of Liver Homogenate

A 10% homogenate of the isolated liver tissue was prepared in Phosphate buffer, pH 7.4 using a Teflon homogenizer in ice-cold condition. The homogenate was centrifuged at 5000 rpm for 10 min. Supernatant collected were used for the determination of non-enzymatic antioxidants reduced glutathione (GSH) and enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD).

Estimation of Antioxidant Enzymes

a) Reduced Glutathione (GSH)

Total reduced glutathione was determined by DTNB method [85].

Principle

The principle involves the reaction of glutathione with Dithio bisnitrobenzoic acid (DTNB), to give a compound which has the absorption maximum at 412 nm.

Procedure

To 1 ml of liver homogenate was precipitated by adding 5% Trichloroacetic acid (TCA) and centrifuged. To 0.5 ml of Phosphate buffer, pH 8.0, 0.5 ml supernatant was added, followed by 2.0 ml of DTNB reagent. The colour developed was read at 412 nm in a Shimadzu UV-160A UV visible recording Spectrophotometer against a blank containing 5% TCA instead of sample. A series of standards were maintained in similar way.

The amount of glutathione was expressed as $\mu\text{g}/\text{mg}$ protein.

b) Catalase

Catalase was assayed according to Kakkar et al [86].

Principle

The breakdown of hydrogen peroxide on addition of enzyme was followed by observing the decrease in light absorption of peroxide solution in the ultraviolet (UV) region.

Procedure

A 3.0 ml reaction mixture containing 1.9 ml buffer, pH 7.0, 1.0 ml of the substrate and 0.1 ml of diluted enzyme was used in this assay. The activity was measured as the change at 240 nm 30 sec interval for 3 minutes in a Shimadzu UV – 160A UV – Visible Recording Spectrophotometer.

The activity was expressed as $\mu\text{mole of H}_2\text{O}_2$ consumed/min/mg protein.

c) Superoxide dismutase (SOD)

Superoxide dismutase was assayed according to Mark Lund method [87].

Procedure

To 0.5 ml of tissue homogenate, 0.5 ml of distilled water was added to dilute the sample. To this 0.25 ml of ice-cold ethanol and 0.15 ml of chloroform were added. The mixture was shaken for a minute at 4⁰ C and then centrifuged. The enzyme activity in the supernatant was determined. Adrenochrome produced in the reaction mixture containing 0.2 ml of EDTA, 0.4 ml of Sodium carbonate and 0.2 ml of epinephrine in a final volume of

2.5 ml was followed at 470 nm in a Shimadzu UV-Vis 160A spectrophotometer. Transition of epinephrine to adrenochrome was inhibited by the addition of the required quality of enzyme. The amount of enzyme required to produce 50% inhibition of epinephrine to adrenochrome transition was taken as one enzyme unit. Activity of the enzyme was expressed as units/min/mg protein.

d) Estimation of Lipid Peroxidation (LPO)

The lipid peroxidation products (as Malonaldehyde) were determined by the Thiobarbituric acid reaction as described by the following method [88].

Procedure

To 0.1 ml of the liver homogenate, 2.0 ml of 20% TCA was added. The contents were mixed well and centrifuged at 4000 rpm for 20 minutes. 2.0 ml of the supernatant was mixed with 2.0 ml of Thiobarbituric acid reagent. Reagent blank standards (5-20 nmoles) were also treated similarly. The contents were heated for 20 minutes in a boiling water bath. The tubes were cooled to room temperature and the absorbance was read at 532 nm in a Shimadzu UV – Visible double beam spectrophotometer. The lipid peroxide content was expressed as moles MDA per 100 mg protein.

6.2.4 Histopathological Studies

After the required amount of liver tissues were utilised for homogenate preparation, the remaining liver tissues were washed with normal saline and fixed in 10% formalin. Paraffin sections were prepared and stained with haematoxylin, eosin thereby examined using light microscopy (x100 magnification).

6.2.5 Statistical Analysis

The data are expressed as mean \pm SEM (n=6). Statistical significance was determined by one way ANOVA followed by Dunnet's test. At 95% confidence interval, p values less than 0.05 were considered significant.

6.3 RESULTS

Effect of MELA on Biochemical Enzyme Levels

There was a significant ($p < 0.05$) increase in the serum hepatic enzyme levels in the group after CCl_4 treatment which was prevented by MELA and indicative of a decrease in serum marker enzyme levels (Table 5) when compared with that of the control group. Pre-treatment with Silymarin and MELA significantly prevented the biochemical changes induced by CCl_4 . The hepatoprotective effect offered by MELA 400mg/kg was found to be greater than that of 200 mg/kg treatment.

Effect of MELA on *In vivo* Lipid Peroxidation

The effect of MELA on localization of radical formation resulting in lipid peroxidation is shown in Table 6. LPO content in the liver homogenate was significantly increased in CCl_4 treated group when compared to normal group ($p < 0.001$). LPO level of MELA 200 and 400 mg/kg and Silymarin treated groups were significantly inhibited by 40%, 90% and 96%, respectively, when compared to the control group.

Effect of MELA on Anti-oxidant Enzyme Levels

The effect of MELA on GSH, SOD and CAT of liver homogenate was shown in the Table 6. The antioxidant enzyme levels in normal control group were observed to be significantly higher than CCl₄ control group. Antioxidant enzyme levels of MELA 200 and 400 mg/kg group were significantly increased by 45 and 90%, respectively, when compared to CCl₄ treated group and Silymarin treated group almost restored the enzyme levels.

Table 6.1 Effect of MELA on Hepatic marker enzymes in CCl₄ induced hepatotoxicity in rats

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Protein (g/dl)	Bilirubin		GGT (IU/L)
					Total (mg/dl)	Direct (mg/dl)	
Vehicle Control	71.5±3.2	74.34± 3.42	194.6±14.3	9.44± 0.02	0.44± 0.03	0.37±0.05	60.4±0.21*
Carbon tetra Chloride treated	232.85±3.29***	162.74±2.63	324.52±15.6	5.95± 0.06	2..92± 0.06*	2.45± 0.04**	80.2±0.18*
MELA (200mg/kg)	92.44 ±3.64***	93.25± 3.5***	206.6± 13.7	7.4± 0.03	0.62± 0.05***	0.69±0.03	62.4±0.31
MELA (400mg/Kg)	74.62±4.01**	75.51± 3.2**	192.6±15.4	8.82 ±0.09	0.45± 0.04	0.53± 0.06**	65.1± 0.29*
Silymarin (100mg/Kg)	79.9± 5.13**	74.41 ± 2.63**	196.4± 16.3	8.74 ± 0.2	0.54 ± 0.02	0.49± 0.04**	56.2±0.27*

Statistical significance was determined by one way ANOVA followed by Dunnet's T test. Values are mean ± SEM expressed as (n=6) P *<0.05; **<0.01; ***<0.001; as compared with CCl₄ induced group
± <0.001 as compared with normal group

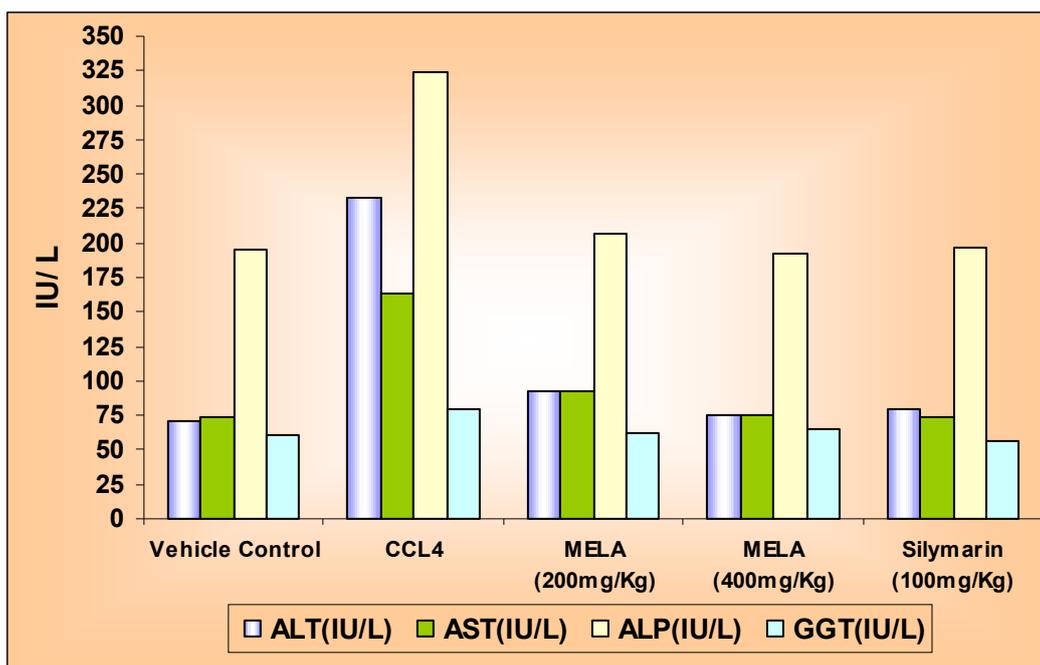


Figure 6.2 Effect of MELA on ALT, AST, ALP, GGT in CCl₄ induced hepatotoxicity in rats

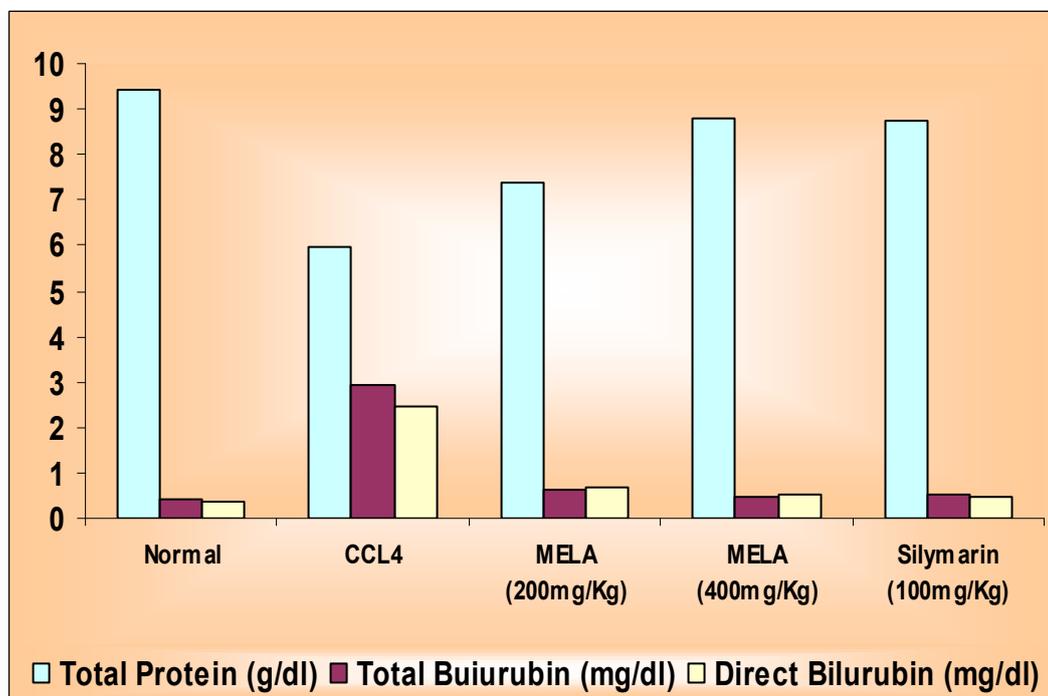


Figure 6.3 Effect of MELA on Protein and Bilirubin level in CCl₄ induced hepatotoxicity in rats

Table 6.2 Effect of MELA on Anti-oxidant enzymes in CCl₄ induced hepatotoxicity in rats

Parameter	GSH	LPO	GPX	CAT	SOD
Control	15.13±1.26***	119.9±21.5	16.89±0.13	35.83±5.43	2.04±0.05
CCl ₄ induced	11.47±0.21*	185.2±11.69*	14.91±0.09	15.73±0.69*	1.79±0.06*
Silymarin 100mg/kg	13.1±0.99*	144.31±6.91**	15.03±0.11*	20.69±0.65**	1.75±.05
MELA 200mg/kg	14.6±1.07**	108.5±10.93***	16.01±0.14***	30.23±0.77***	1.99±0.13*
MELA 400mg/kg	15.09±1.11***	120.2±10.02***	16.87±0.15***	34.31±0.79***	2.02±0.08**

Statistical significance was determined by one way ANOVA followed by Dunnet's T test.

Values are mean ± SEM expressed as (n=6)

P *<0.05; **<0.01; ***<0.001; as compared with CCl₄ induced group
+<0.001 as compared with normal group.

LP-Lipid peroxidation [nmoles of malonaldehyde (MDA) formed/mg protein/h)

GPX - Glutathione peroxidase (µg of glutathione consumed/min/mg protein);

CAT - Catalase (unit/min/mg protein)

SOD - Superoxide dismutase (unit/min/mg protein)

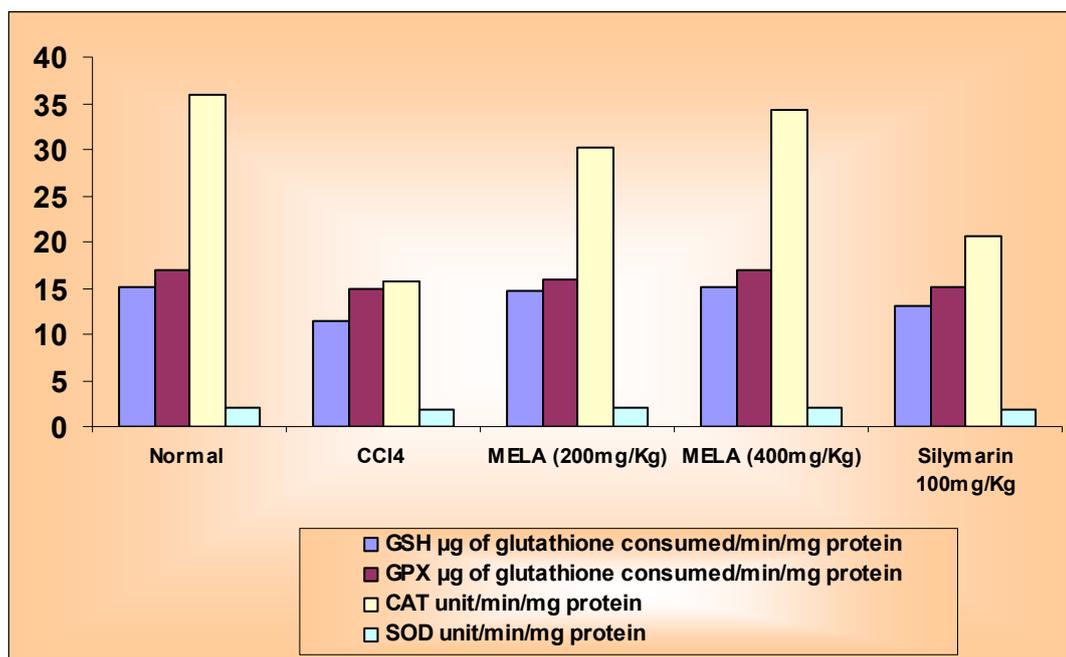


Figure 6.4 Effect of MELA on Antioxidant enzymes in CCl₄ induced hepatotoxicity in rats

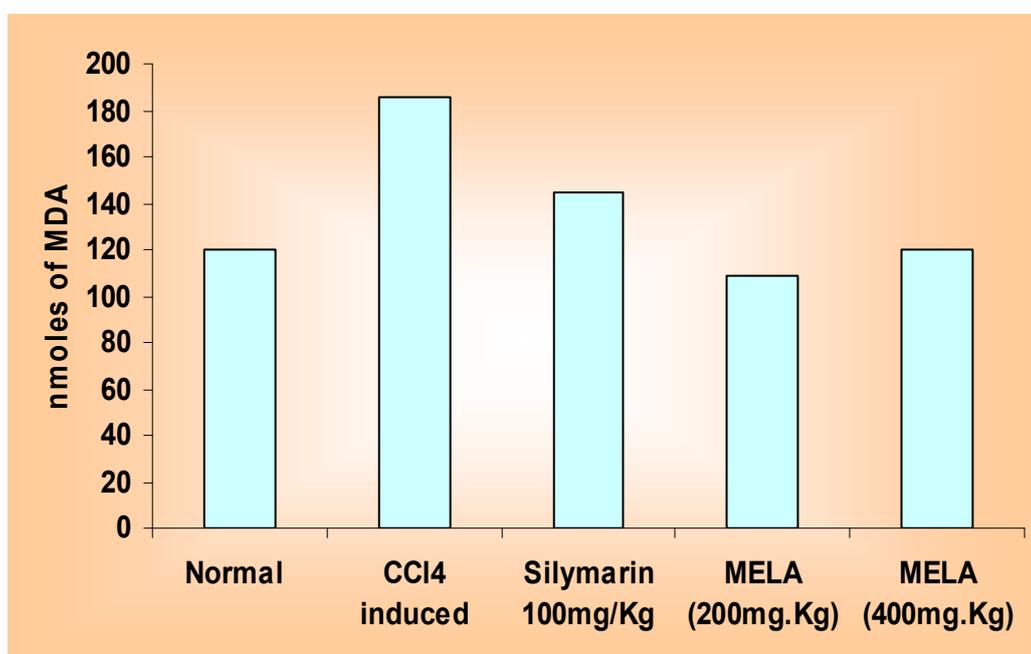


Figure 6.5 Effect of MELA on Lipid Peroxidation in CCl₄ induced hepatotoxicity in rats

Effect of MELA on Histopathological Studies

Histopathological evaluation of control group as shown in (Figure 6.6(a)) and CCl₄ treated rats revealed fatty degeneration, necrosis and fibrosis (Figure 6.6(b)). Concurrent administration of MELA preserved the histological structure of the liver though there was a mild congestion and regeneration of liver tissues (Figure 6.6(c) and 6.6(d)).

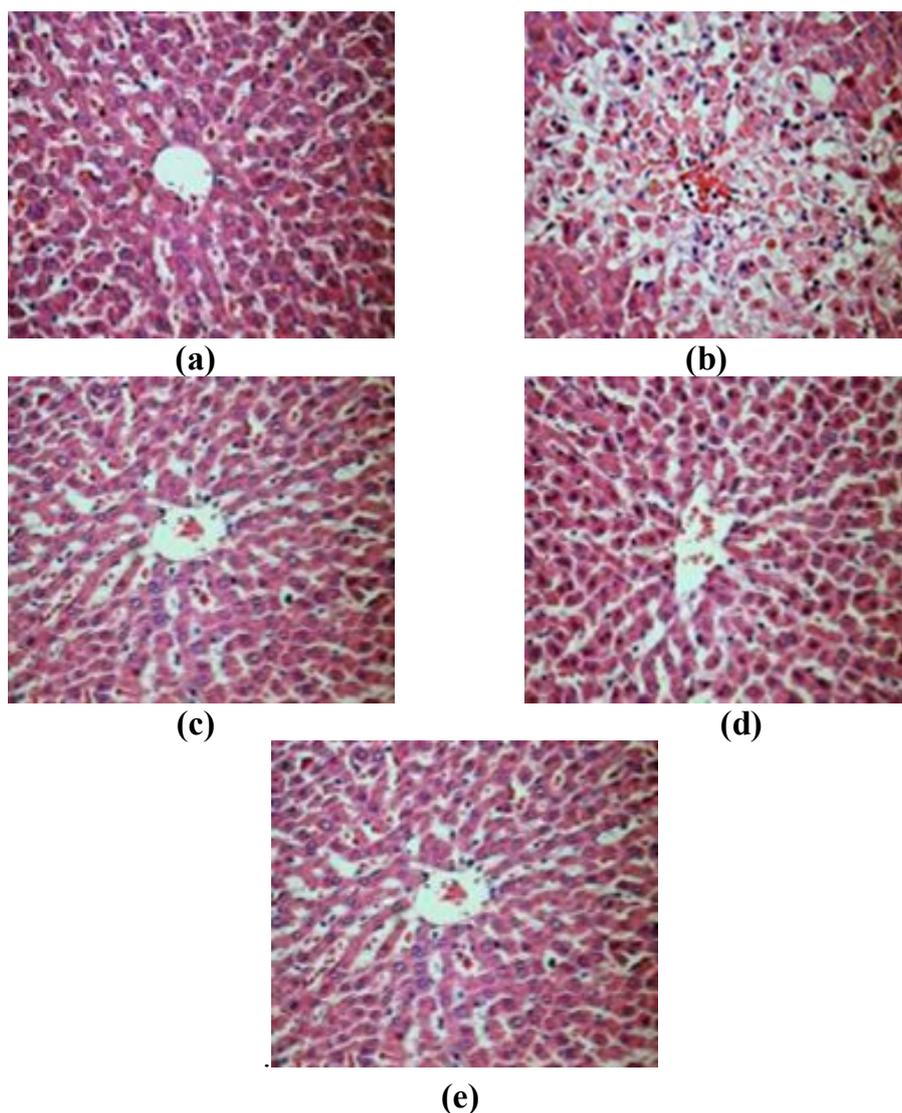


Figure 6.6 Histopathology of liver sections of animals (x100)

- (a) Control group (received distilled water for 10 days) showing normal architecture of hepatic cells.
- (b) CCl_4 (0.5ml/kg i.p for 3 days) treated group showing centrilobular degeneration, necrosis of hepatic cells.
- (c) CCl_4 (0.5ml/kg i.p for 3 days) + MELA 200 mg/kg (for 10 days) showing mild degeneration and reverting to regeneration.
- (d) CCl_4 (0.5ml/kg i.p for the last 3 days) + MELA 400 mg/kg (for 10 days) showing complete regeneration and almost normal architecture of hepatocytes.
- (e) CCl_4 (0.5ml/kg i.p for 3 days) + Silymarin 100 mg/kg (for 10 days) showing complete regeneration and normal architecture of hepatocytes.

6.4 DISCUSSION

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases. It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as Reactive oxygen species (ROS). The high reactivity of ROS may trigger a host of disorders in body resulting in tissue damage and necrosis in many instances [89].

CCl_4 – mediated hepatotoxicity was taken here as the experimental model for liver injury. Carbon tetrachloride is the most commonly used hepatotoxins in the experimental study of free radical induced liver diseases [90]. The hepatotoxicity of CCl_4 is due to the metabolic formation of the highly reactive trichloromethyl free radical which attacks the polyunsaturated fatty acids of the membrane of the endoplasmic reticulum and initiates a chain reaction leading to the formation of lipid peroxides. The lipid per oxidative degeneration of bio membrane is one of the principle causes of hepatotoxicity of CCl_4 [91] which induces hepatic microsomal enzyme systems and vice versa by antioxidants which mop up the free radicals.

In the assessment of liver damage by hepatotoxins like CCl_4 , the determination of enzyme levels such as AST and ALT is largely used. This is evidenced by an elevation in the serum marker enzymes namely AST, ALP and ALT. At the two dose levels (200 and 400 mg/kg), a significant reduction of the enzyme levels in a dose dependant manner and an increase in the level of TP by MELA indicate hepatoprotective activity as stimulation of protein synthesis accelerates the regeneration process and production of liver cells.

Elevation of total bilirubin which results from decreased uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while

increased levels of direct or conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts [92]. A reduction of increase in total and conjugated bilirubin by *L.acidissima* extract further proves its protective effect against CCl₄ induced liver toxicity. The extract perhaps protects the liver cell from damage, thereby enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts. These reports from the study show that the methanolic extract of *L.acidissima* possess hepatoprotective activity as demonstrated.

The body has an effective mechanism to prevent and neutralize the free radical – induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, and GPX etc. When the balance between ROS production and antioxidant defences is lost, ‘oxidative stress’ results, which through a series of events deregulate the cellular functions leading to various pathological conditions. Carbon tetrachloride induced liver damage simulates free radical induced damage [93]. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

In the present study, elevated level of TBARS observed in CCl₄ treated rats indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. TBARS produced as by-products of LPO that occurs in hydrophobic core of bio-membranes [94]. The significant decline in the concentration of these constituents in the liver tissue of CCl₄ and MELA 400mg/kg administered rats indicates better anti-lipid peroxidative effect of *L.acidissima* than 200mg/kg treated animals.

GSH is a major non-protein thiol in living organisms which plays a central role in coordinating the body’s antioxidant defence processes. Perturbation of GSH status of a biological system has been reported to lead serious consequences. Decline in GSH content in the liver of CCl₄-

intoxicated rats, and its subsequent return towards near-normalcy in CCl₄ and MELA(200 and 400 mg/kg) treated rats reveal the antioxidant effect of the extract. Explanations of the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSH depletion and destruction of free radicals [95]. These two factors are believed to attribute to the hepatoprotective properties of the fruit pulp of *L.acidissima*

SOD, CAT and GPX constitute a mutually supportive team of defence against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady-state level of O₂. CAT is a heme protein, localized in the peroxisomes or the micro peroxisomes. These enzyme catalyses the decomposition of H₂O₂ to water and oxygen and thus protecting the cell from oxidative damage by H₂O₂ and OH—. GPX is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydro peroxides with reduced glutathione to form glutathione di sulphide (GSSG) and the reduction product of the hydro peroxide. In our study, decline in the activities of these enzymes in CCl₄- administered rats revealed that LPO and oxidative stress elicited by CCl₄ –intoxication have been nullified due to the effect of *L.acidissima*. Since MELA treated groups has significantly elevated the GSH, SOD, CAT contents of the liver against CCl₄ intoxication, it may be helpful in treating the hepatotoxicity induced by free radicals[96].

Estimation of γ -GGT levels is a valuable parameter with a high negative predictive value for liver diseases. The γ -GGT levels increase proportionately with the increase in microsomal enzymes. There is usually a severe damage to tissue membrane at CCl₄ toxic doses because γ -GGT is a membrane bound enzyme. But the γ -GGT levels have significantly decreased in MELA treated groups as shown in the Table 6.1 which is indicative of the membrane stabilizing activity of MELA in improvement of liver function.

In the histopathological studies, the first cells to be damaged are those in the centrilobular region where microsomal enzyme activity is the greatest. The initial damage produced is highly localised in the endoplasmic reticulum which results in loss of cytochrome p450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver and necrosis, a characteristic of CCl₄ poisoning. If the damage is severe, it leads to disturbances in the water and electrolyte balance of hepatocytes leading to an abnormal increase in liver enzymes in plasma, there by impairing mitochondrial functions, followed by hepatocellular necrosis. The reverse of this phenomenon can be considered as the index of hepatoprotective activity [97].

Histopathological studies of liver sections has shown that the pre-treatment with MELA exhibited protection against CCl₄ induced fatty degeneration and necrosis of the liver tissue, confirming the results of biochemical studies and indicating the hepatoprotective properties of MELA. The improved histology of the liver as seen in histopathological observations on animals pre-treated with the plant material as compared to that seen in animals administered only CCl₄ indicated the possibility of the plant material *L.acidissima* being able to induce accelerated regeneration of the liver.

6.5 CONCLUSION

The present study reveals that hepatoprotective and antioxidant activities of MELA in experimental animal model against CCl₄ induced hepatotoxicity which was proved by the serum marker enzymes and the antioxidant enzyme levels. Further works are being carried out to isolate and identify the active principle involved in the hepatoprotective and antioxidant activities of this plant extract.