CHAPTER III

RESULTS

The present investigation was aimed to assess the hepatoprotective activity of *Eclipta alba* and *Boerhavia diffusa* extracts, both *in vitro* and *in vivo* conditions, against carbon tetrachloride (CCl₄) - induced toxicity in mice. As a whole, the study is divided in three parts: **Part I:** Phytochemical analysis of the plant, **Part II:** To study ameliorative effect of *Eclipta alba* and *Boerhavia diffusa* plant extracts against CCl₄- induced toxicity: an *in vitro* study, and **Part III:** Mitigatory effects of *Eclipta alba* and *Boerhavia diffusa* extracts against CCl₄ - induced toxicity in mice: an *in vivo* study.

PART I

**Phytochemical analysis of the plants**

Both *Eclipta alba* and *Boerhavia diffusa* extracts were analysed for their phytochemical components, antioxidative potency and their active constituents.

**(A) Qualitative and quantitative analysis**

**Yield:** Percent yield of hydro-alcoholic extracts of *Eclipta alba* and *Boerhavia diffusa* were 15.03 ± 1.38 and 10.89 ± 1.05 gm% respectively (Table 3.1). As shown in table 3.1 both the extracts were found to contain considerably large amount of crude phytochemicals as mentioned below:

**Qualitative analysis**

Table 3.1 depicts the results of phytochemical analysis of *Eclipta alba*/*Boerhavia diffusa* extracts. The qualitative analysis of *Eclipta alba*/*Boerhavia diffusa* extracts.
diffusa extracts indicated the presence of phenolics, flavonoids, tannins and ascorbic acid contents, which were later determined quantitatively using standard methods.

Quantitative analysis

Total phenolic content:

Total phenolic content (TPC) of both the extracts were estimated using standard method. The concentration of TPC determined in hydro-alcoholic extract of Eclipta alba was 98.39 mg gallic acid equivalent/gm dry weight calculated using equation that was obtained from standard gallic acid graph. However, phenolic content of hydro-alcoholic extract of Boerhavia diffusa was 70.80 mg gallic acid equivalent/gm dry weight (Table 3.1).

Flavonoid content:

Quantification of flavonoid content showed that hydro-alcoholic extract of Eclipta alba contained 86.53 mg quercetin equivalent/gm dry weight of extract which was only 35.91 mg quercetin equivalent/gm dry weight of Boerhavia diffusa extract (Table 3.1).

Tannin content:

Standard curve for tannin estimation was plotted using various concentrations of rutin. Tannin content of hydro-alcoholic extract of Eclipta alba was found to be 40 mg rutin equivalent/gm dry weight, while Boerhavia diffusa extract contained only 25.71 mg rutin equivalent/gm dry weight of tannin (Table 3.1).

Ascorbic acid content:

Ascorbic acid content of hydro-alcoholic extract of Eclipta alba was found to be 2.24 µg/gm dry weight of extracts; however, ascorbic acid content in Boerhavia diffusa extract was only 0.67 µg/gm dry weight (Table 3.1).
(B) **Antioxidative potency**

This study was designed to assess the potential of *Eclipta alba* and *Boerhavia diffusa* extracts to scavenge free radicals using chemical models.

**Superoxide radical scavenging assay:**

Superoxide radicals generated from PMS/NADH-NBT system were strongly scavenged by various concentrations of *Eclipta alba* and *Boerhavia diffusa* extracts. The decrease in colour intensity was observed with increasing concentration of extracts indicating consumption of the radicals in the reactions mixture (Fig. 3.1). Maximum effect was achieved at 300 µg/mL concentration of the extracts. Maximum scavenging effect found with *Eclipta alba* extract was 84.19% while it was 81.91% in case of *Boerhavia diffusa* extract. The effect was concentration-dependent for both the extracts ($R^2 = 0.9874, 0.9932$ respectively). Concentration required to scavenge 50% ($IC_{50}$) of the radicals was 150 µg/mL for *Eclipta alba* and extract and *Boerhavia diffusa* 150 µg/mL extract.

**Hydroxyl radical scavenging assay:**

Hydroxyl radical scavenging capacity of extract is directly proportional to its antioxidative potency. The percent inhibition of hydroxyl radical increased significantly (p<0.05) with increasing concentrations of hydro-alcoholic extracts of *Eclipta alba* and *Boerhavia diffusa* (Fig. 3.2). Results indicated that *Eclipta alba* extract was more potent than *Boerhavia diffusa* extract; maximum protection for *Eclipta alba* was (71.84%), which was 69.06% in case of *Boerhavia diffusa* extract. The protective effect was concentration-dependent ($R^2 = 0.9956, 0.9700$) and was highest at 50 µg/mL concentration (Fig. 3.2). $IC_{50}$ values of *Eclipta alba* and *Boerhavia diffusa* extracts were 30 and 40 µg/mL respectively.
**Nitrous oxide radical scavenging assay:**

Nitrous oxide radicals generated from sodium nitroprusside at physiological pH were significantly (p<0.05) inhibited by *Eclipta alba* and *Boerhavia diffusa* extracts. Percent inhibition was concentration-dependent ($R^2 = 0.9035, 0.8948$) and maximum at 250 µg/mL concentration of the extract. Highest scavenging effect found with *Eclipta alba* extract was 85.55%, whereas it was 81.65% in case of *Boerhavia diffusa* extract (Fig. 3.3). IC$_{50}$ values for nitrous oxide scavenging activity were 50 µg/mL for *Eclipta alba* and *Boerhavia diffusa* extracts.

**DPPH radical scavenging assay:**

DPPH radical scavenging activity of various concentrations of *Eclipta alba* and *Boerhavia diffusa* were found statistically significant (p<0.05). Decrease in absorbance due to antioxidative effect of soluble solids of *Eclipta alba* and *Boerhavia diffusa* was highest at 250 µg/mL concentration (Fig. 3.4). *Eclipta alba* was found to be more potent (82.47%) than *Boerhavia diffusa* (80.00%). Scavenging effect of both the extracts were concentration-dependent ($R^2 = 0.8216, 0.9146$). IC$_{50}$ value for *Eclipta alba* extract was 50 µg/mL, while it was 100 µg/mL for *Boerhavia diffusa* extract.

**Reducing ability:**

The presence of reductant (antioxidant) in the tested extracts of *Eclipta alba* and *Boerhavia diffusa* resulted in the reduction in Fe$^{3+}$/ferricyanide complex to ferrous form (Fe$^{2+}$). Increasing concentrations of the extracts resulted in simultaneous increase of reducing power (Fig. 3.5). Highest reducing ability was found with *Eclipta alba* extract (75.59%), whereas it was 72.12% in case of *Boerhavia diffusa* extract at 250 µg/mL concentration (Fig. 3.5). Increase in reducing ability of the extracts was
concentration-dependent ($R^2 = 0.9863, 0.9842$). IC$_{50}$ values for reducing ability were 100 and 150 µg/mL for *Eclipta alba* and *Boerhavia diffusa* extracts respectively.

**Fe$^{+2}$ chelating activity:**

Ferrozine - Fe$^{+2}$ complex produces violet colour in the reaction mixture in presence of metal ions which was significantly reduced by addition of *Eclipta alba* and *Boerhavia diffusa* extracts. Formation of coloured chromophore is interrupted in the presence of chelating agents of *Eclipta alba* and *Boerhavia diffusa* extracts and resulted in decreased optical density (Fig. 3.6). Maximum inhibition achieved with *Eclipta alba* extract was 78.77% which was 71.08% in case of *Boerhavia diffusa* extract. Both the extracts chelated metal ions in a concentration – dependent manner ($R^2 = 0.9656, 0.9862$). IC$_{50}$ values for *Eclipta alba* and *Boerhavia diffusa* extract was 100 and 150 µg/mL respectively.

**(C) Identification and quantification of active components of plant extracts**

Standardization of plant extracts were done by quantifying the major active components of both the plant extracts. The major active component present in these two plants namely wedelolactone from *Eclipta alba* and boerhavinone B from *Boerhavia diffusa* were separated from other constituents by reverse phase HPLC analysis.

Under the optimized chromatographic conditions, the reaction time for standard wedelolactone was 2.223 as shown in Fig.3.7. Chromatogram of hydro-alcoholic extract of *Eclipta alba* showed five peaks of 1.627, 2.233, 2.515, 2.833 and 3.068 min as shown in Fig. 3.8. The peak of 2.233 in the crude extract correspond to wedelolactone, which was confirmed by spiking the sample with standard stock
solution of wedelolactone (1000 µg/mL) as depicted in Fig. 3.8. Percent concentration of wedelolactone present in *Eclipta alba* extract was 4.47 %.

Under the optimized chromatographic condition the retention time for standard boerhavinone B was 2.250 as shown Fig. 3.9. Chromatograph of hydro-alcoholic extract of *Boerhavia diffusa* showed four peaks of 1.622, 2.252, 2.598 and 4.329 min as shown Fig.3.10. The peak of 2.252 in the crude extract corresponds to boerhavinone B, which was confirmed by spiking the sample with standard stock solution of boerhavinone B (1000 µg/mL) as depicted in Fig. 3.10. Percent concentration of boerhavinone B present in *Boerhavia diffusa* extract was 0.194 %.

**PART II**

To study ameliorative effect of *Eclipta alba* and *Boerhavia diffusa* plant extracts against CCl₄-induced toxicity: an *in vitro* study

*Lipid peroxidation:*

Exposure of liver homogenates with various concentrations (5-50 µg/mL) of CCl₄ had resulted in significant (p<0.05) increase in MDA levels-marker of lipid peroxidation (Fig. 3.11) as compared to control. Maximum increased in lipid peroxidation was at 10 µg/mL concentration of CCl₄ (Table 3.2).

Cotreatment of CCl₄ (10 µg/mL) along with various concentrations (25-150 µg/mL) of *Eclipta alba*/*Boerhavia diffusa* extracts as well as Liv. 52 extracts to the liver homogenates had significantly reduced levels of CCl₄-induced lipid peroxidation (Table 3.3). The effect was concentration-dependent; however, *Boerhavia diffusa* extract was less potent as compared to *Eclipta alba* extract. Similarly, Liv. 52 cotreatment to CCl₄ – treated liver homogenates had also reduced level of lipid
peroxidation significantly (p<0.05) in a concentration-dependent manner (r = 0.9929).

However, the protective effect of *Eclipta alba* / *Boerhavia diffusa* was higher as compared to Liv. 52 - treated liver homogenate. The maximum protection achieved by *Eclipta alba* (97.44%), *Boerhavia diffusa* (95.80%) and Liv. 52 (94.00%) was at 150 µg/mL concentration. The protection given by 25, 50, 75, 100 and 150 µg/mL concentration of *Eclipta alba* extract was 24.58%, 44.33%, 73.81%, 83.32%, 91.75% and 97.44% respectively. In a same manner, *Boerhavia diffusa* extract and Liv. 52 at concentration of 25, 50, 75, 100 and 150 µg/mL showed protective effect of 14.03%, 35.96%, 58.96%, 77.28%, 86.13% and 95.80% as well as 7.27%, 29.79%, 48.97%, 68.94%, 80.60% and 94.00% respectively (Fig. 3.13).

**Protein content:**

Table 3.2 shows the effect of CCl₄ on protein content in liver homogenate. Exposure of liver homogenates with various concentrations of CCl₄ (5, 10, 20, 30, 40 and 50 µg/mL) had resulted in significant (p<0.05) decrease in protein content in a concentration-dependent (r = 0.9926) manner as compared to control (Table 3.2). The percent decrease in protein content was 30.45%, 37.36%, 46.86%, 53.92%, 60.28% and 67.03% at 5, 10, 20, 30, 40, 50 µg/mL concentrations respectively (Fig. 3.12).

Results shown in Table 3.3 indicate effect of cotreatment of CCl₄ and plant extracts on protein content in liver homogenates. Concurrent addition of extracts of *Eclipta alba* / *Boerhavia diffusa* as well as Liv. 52 along with CCl₄ (10 µg/mL) had resulted into significant [(p<0.05) only for 100 from 100 µg/mL to 150 µg/mL)] rise in protein content in a concentration-dependent (r = 0.9948, 0.9923, 0.9903 respectively) manner as compared with CCl₄ alone treated liver homogenates. Addition of *Eclipta alba* / *Boerhavia diffusa* / Liv. 52 at 25, 50, 75, 100, 125, 150 µg/mL concentration in CCl₄ (10 µg/mL) - treated liver homogenates showed
significant increase in protein content \([Eclipta\ alba\ (6.55\%\ 10.73\%,\ 17.63\%,\ 20.18\%,\ 25.33\%\ and\ 30.22\%)]/\ Boerhavia\ diffusa\ (3.85\%,\ 8.58\%,\ 13.25\%,\ 18.45\%,\ 25.61\%\ \text{and}\ 29.17\%)/\ Liv.\ 52\ (2.44\%,\ 3.50\%,\ 9.31\%,\ 14.27\%,\ 21.97\%\ \text{and}\ 27.76\%)].\] Protecive effect was highest for \(Eclipta\ alba\), followed by \(Boerhavia\ diffusa\) and Liv. 52 (Fig. 3.14).

**PART III**

**Mitigatory effect of \(Eclipta\ alba\) and \(Boerhavia\ diffusa\) plant extracts on \(CCl_4\)-induced liver toxicity in mice: \textit{in vivo} study**

**Acute oral toxicity study:**

Acute oral toxicity study of hydro-alcoholic extract of \(Eclipta\ alba\) and \(Boerhavia\ diffusa\) showed absence of any mortality at the dosage of 2000 mg/kg body weight. No other clinical or behavioural changes were observed in extract-treated animals (Table 3.4).

**Clinical symptoms:**

No treatment related mortality was recorded in any of the groups. Oral administration of \(CCl_4\) (826 mg/kg body weight/day) for 30 days caused lethargy, dullness, decreased in feed intake and reduction in body hair in mice. However, cotreatment of \(Eclipta\ alba/Boerhavia\ diffusa\) plant extracts at three different doses (100, 200 and 300 mg/kg body weight/day) along with \(CCl_4\) in mice resulted in significant reduction in symptoms of lethargy, dullness, increased in feed intake and normal body weight in a dose-dependent manner.
**Body weight:**

Table 3.5 shows the results of plant extracts treatment on CCl$_4$-induced changes in the body weight of mice. No significant difference in body weight was observed between different control groups (Groups 1-5). Carbon tetrachloride treatment for 30 days (Group 6) caused, as compared to vehicle control (Group 2), a significant (p<0.05) reduction in body weight (27.76%).

Oral administration of CCl$_4$ along with hydro-alcoholic extract of *Eclipta alba* (Group 7-9)/ *Boerhavia diffusa* (Group 10-12)/ Liv. 52 (Group 13) caused significant (p<0.05) increase in body weight as compared to CCl$_4$ alone treated animals (Group 6) in a dose-dependent manner (EA: $r = 0.9767$, BD: $r = 0.9901$). The percent increase in body weight at three different doses of *Eclipta alba*/*Boerhavia diffusa* extracts were 8.68% (EA100), 13.57% (EA200), 22.68% (EA300) and 3.20% (BD100), 10.37% (BD200), 20.13% (BD300). The 300 mg/kg body weight dose was the most effective in both plant extracts as evident by the percent protection. The hepatoprotective efficacy was higher in case of *Eclipta alba* - treated groups as compared to that of *Boerhavia diffusa* - treated animals.

The result was also compared with standard drug Liv. 52 - treated group of mice (Group 13) and the protective effect was higher in both plant extracts as compared to Liv. 52 - treated group of animals (Group 13) (Fig. 3.15).

**Absolute and relative liver weights:**

No treatment related clinical signs were observed in control groups (Groups 1-5). However, enlargement of liver with fatty, pale, pitted with coarsely granular surfaces in CCl$_4$ – treated animals was found to reduce with the administration of three different doses of *Eclipta alba*/*Boerhavia diffusa* plant extracts in a dose-dependent manner. The most effective dose was 300 mg/kg body weight in both plant
extracts treated groups and protective effect was highest in *Eclipta alba* followed by *Boerhavia diffusa* extract and Liv. 52.

Table 3.6 shows the results of the plant extract treatments on CCl₄-induced changes in the absolute and relative liver weights of mice. No significant difference was observed in absolute and relative weights of liver in different control groups (Groups 1-5). Carbon tetrachloride treatment, as compared to vehicle control, caused a significant (p<0.05) increase in absolute (35.14%) and relative (80.52%) liver weights of mice (Fig. 3.16 and 3.17 respectively).

Oral administration of *Eclipta alba*/*Boerhavia diffusa* extract in three different doses along with CCl₄ (Groups 7–9, 10–12) significantly (p<0.05 only mid and high dose group) mitigated CCl₄-induced changes in absolute weight of liver (Group 6). Hepatoprotective percentage for 100, 200 and 300 mg/kg body weight/day treated groups (Groups 7-9, 10-12) for absolute liver weight was comparatively higher for *Eclipta alba* (40.38%, 71.15%, 96.15%) than that of *Boerhavia diffusa* (28.85%, 63.46%, 88.46%) (Fig. 3.16). The protective effect of both the plant extracts were in a dose-dependent manner (EA: r = 0.9982, BD: r = 0.9957). Amelioration was more at 300 mg/kg body weight dose in both the plant extract treated groups of mice.

Co-administration of *Eclipta alba*/*Boerhavia diffusa* extracts along with CCl₄ significantly ameliorated CCl₄-induced rise in relative liver weight. Hepatoprotective index was calculated for *Eclipta alba*/*Boerhavia diffusa* extract - treated groups of mice (Group 7-9/10-12). Hepatoprotective index for *Eclipta alba* treated groups (Group 7-9) were 41.66% (EA100), 67.47% (EA200), 93.82% (EA300) for relative liver weight. In a similar manner, hepatoprotective index for *Boerhavia diffusa* extracts treated groups (Group 10-12) were 25% (BD100), 41.66% (BD200), and 87.37% (BD300). Results revealed that hepatoprotective effect of both the plant
extracts were in a dose-dependent manner (EA: \( r = 1 \) and BD: \( r = 0.9997 \)) and highest protection was achieved at 300 mg/kg body weight dose treated groups of mice (Group 9 and 12) (Fig. 3.17).

The result was also compared with the Liv. 52-treated group (Group 13). The hepatoprotective effect of both plant extracts was significantly higher as compared to Liv. 52-treated group. Hepatoprotective index was found to be highest for *Eclipta alba* followed by *Boerhavia diffusa* and Liv. 52.

**Histopathological examinations**

**Hematoxylin-eosin staining:**

Liver sections (H & E stained) of untreated control (Plate A) and vehicle control mice (Plate B) showed normal arrangement of hepatocytes with clearly visible nucleus, sinusoids, central vein and portal triad. Oral administration of *Eclipta alba*/*Boerhavia diffusa* extracts/ Liv. 52 alone treatment did not cause any alteration in normal architecture of hepatocytes and central vein (Plate C; Plate D and Plate E).

Oral administration of CCl\(_4\) for 30 days caused severe hepatocellular necrosis, cytoplasmic vacuolization, fatty infiltration like ballooning of hepatocytes, fibrosis, lymphocytic infiltration and loss of cellular boundaries (Plate F).

Cotreatment of three different doses of *Eclipta alba*/*Boerhavia diffusa* extracts along with CCl\(_4\) caused amelioration of CCl\(_4\) – induced histopathological changes in a dose-dependent manner (*Eclipta alba*: G – I; *Boerhavia diffusa* J – L). Results revealed complete restoration of the normal architecture and arrangement of hepatocytes in mice administered with *Eclipta alba* (300 mg/kg body weight/day) along with CCl\(_4\) (Plate I). The radial arrangement of hepatocytes became prominent. The inflammatory and fatty degenerative changes were completely absent (Plate I).
300 mg/kg body weight) along with CCl₄ caused significant, dose-dependent protection against the changes induced by CCl₄. Oral administration of Boerhavia diffusa (300 mg/ kg body weight/day) along with CCl₄ resulted in restoration of normal liver architecture (Plate L).

Results were compared with Liv. 52 (300 mg/kg body weight/day) treated group of animals (Group 13). Liv.52 administration along with CCl₄ caused significant protection against the changes induced by the CCl₄ but to a lesser extent as compared to Eclipta alba/ Boerhavia diffusa - treated groups of mice (Plate M). On the basis of histopathological studies it could be concluded that Eclipta alba treatment completely prevents CCl₄-induced hepatotoxicity in mice, very closely followed by Boerhavia diffusa.

**Masson’s trichrome staining:**

Masson’s trichrome staining for collagen in liver sections of untreated control and vehicle control groups (Group 1 and 2) (Plate N and O) showed normal histological architecture with normal hepatocytes and central vein. No deposition of collagen (blue area) was observed around blood vessels in untreated and vehicle control groups (Group 1 and 2) (Plate N and O). Oral administration of Eclipta alba/ Boerhavia diffusa extracts/ Liv. 52 alone treatment did not cause any alteration in normal architecture of hepatocytes and central vein was observed (Plate P, Q and R). However, the liver of mice treated with CCl₄ for 30 days showed pronounced destruction of the liver architecture with extensive accumulation of connective tissue resulting in severe fibrosis between the central and portal veins and nodules (Plate S).

Cotreatment of Eclipta alba (Plate T) along with CCl₄ resulted in restoration of normal liver architecture. Fibrotic changes were ameliorated in the Eclipta alba extract - treated mice. Similarly, administration of Boerhavia diffusa extract (Plate U)
along with CCl₄ resulted in decreased accumulation of collagen and the liver architecture was restored to near normalcy. The anti-fibrotic potential was found to be higher in *Eclipta alba* extract - treated group as compared to *Boerhavia diffusa* extract – treated group of mice.

The results were compared with the standard polyherbal drug Liv. 52 - treated group. The mitigatory effect of plant extracts was higher as compared to Liv. 52 - treated group of mice (Group 13) (Plate V).

**LIVER BIOCHEMICAL ANALYSIS**

*Protein, carbohydrate, nucleic acid and lipid contents:*

Table 3.7 present the results of protective effect of plant extracts on biochemical changes induced by CCl₄ in liver of mice. No significant change was observed in protein, glycogen, DNA and RNA contents in different control groups (Groups 1 - 5). Oral administration of CCl₄ (Group 6) caused significant (p<0.05) decrease in protein (-48.17%), glycogen (-66.30%), DNA (-36.83%) and RNA (-47.16%) contents in liver of mice.

Oral administration of *Eclipta alba*/*Boerhavia diffusa* extracts along with CCl₄ significantly (p<0.05) restored protein content in a dose-dependent (EA: 1 and BD: 0.9973) manner (Table 3.7). The hepatoprotective effect of plant extracts at three different doses of 100, 200 and 300 mg/kg body weight on CCl₄-induced changes in total protein content was 43.12%, 68.32 and 92.82% in case of *Eclipta alba* and 29.36%, 50.31% and 66.50% in case of *Boerhavia diffusa* treatments respectively (Fig. 3.18).

Glycogen content was also significantly (p<0.05) restored back to the normal in a dose-dependent manner (EA: r = 0.9961, BD: r = 0.9930). The hepatoprotective index for glycogen content was 18.34%, 48.34% and 89.71% in *Eclipta alba* - treated
groups of mice (Group 7-9) and 16.67%, 40.84% and 77.50% in *Boerhavia diffusa* - treated groups (Groups 10-12) at three different doses (Fig. 3.19).

Oral administration of *Eclipta albal* *Boerhavia diffusa* extracts, significantly (p<0.05) restored CCl₄-induced changes in DNA and RNA contents. Hepatoprotective index of *Eclipta alba* extract was [DNA (EA100: 20.11%, EA200: 37.03%, EA300: 75.94%) and RNA (EA100: 29.29%, EA200 60.57%, EA300 87.50%)] comparatively higher than that of *Boerhavia diffusa* extract [DNA (BD100: 14.93%, BD200: 37.03%, BD300 75.94%), RNA (BD100 18.79%, BD200 44.42%, BD300 74.38%)] in liver of mice (Fig. 3.20 and 3.21 respectively) (Table 3.7). The effect was significant (p<0.05) and dose-dependent (EA: r = 0.9971, 0.9876 and BD: r = 0.9991, r = 0.9990) in DNA and RNA content respectively. The results were compared with standard drug Liv. 52 - treated group of mice and the protective effect was lesser as compared to *Eclipta albal* *Boerhavia diffusa* - treated groups of mice (Group 13).

Table 3.8 present the results of mitigatory effect of plant extracts on CCl₄-induced changes in the lipid contents in liver of mice. No significant changes were observed in total lipid and cholesterol contents amongst different control groups (Group 1-5). Contents of total lipid and cholesterol were significantly (p<0.05) increased with CCl₄ treatment. As compared with the vehicle control, the increase in total lipid and cholesterol contents were 95.50% and 91.01% respectively.

Oral administration of three different doses of *Eclipta albal* *Boerhavia diffusa* extract along with CCl₄ caused significant (p<0.05) and dose-dependent (EA: r = 0.9912, 0.9919, BD: 0.9845, 0.9985) decrease in total lipid and cholesterol contents respectively. As indicated by hepatoprotective index, three different doses of *Eclipta albal* *Boerhavia diffusa* extract mitigated CCl₄-induced increased in total lipid content by 32.25% (EA100), 56.89% (EA200), 96.38% (EA300) and 22.47% (BD100),
42.40% (BD200), 80.08% (BD300) (Table 3.8, Fig. 3.22). Also the increased cholesterol content was significantly ameliorated by *Eclipta alba*/*Boerhavia diffusa* extract treatment for 30 days (EA100: 46.92%, EA200: 74.08%, EA300: 91.36% and BD100: 28.40%, BD200: 56.80%, BD300: 80.25%) (Fig. 3.23).

The effect of plant extracts were compared with the standard drug Liv. 52 (300 mg/kg body weight/day) where hepatoprotective index was 71.74% in case of total lipid content and 66.67% in case of cholesterol content. The effects of both the plant extracts were higher as compared to Liv. 52 - treated group of mice (Group 13). Maximum protection was achieved with 300 mg/kg body weight dose of *Eclipta alba* and *Boerhavia diffusa* extract (Group 9 and 12) (Fig. 22 and 23).

**HEPATIC ENZYMATIC ALTERATIONS**

*Activities of ALT, AST, ALP and ACP in liver:*

Table 3.9 shows the results of CCl4-induced changes in the activities of some enzymes in liver of mice. No significant changes in the enzyme activities were found between untreated control, vehicle control and antidote control groups (Group 1-5). Oral administration of CCl4 (Group 6) for 30 days had significantly increased the activities of ALT, AST, ALP and ACP in liver. The percent increase in the activities of ALT, AST, ALP and ACP were 477.78%, 375.66%, 90.32%, 109.86% respectively.

Oral administration of *Eclipta alba*/*Boerhavia diffusa* extract ameliorated CCl4-induced changes in enzyme activities. The effect was dose-dependent in *Eclipta alba*/*Boerhavia diffusa* extract - treated groups (ALT: r = 0.9986 and 0.9955, AST: r = 0.9999 and 1, ALP: r = 0.9887 and 0.9859, ACP: r = 0.9926 and 0.9998) and significant (p<0.05) as compared to toxin - treated group. Maximum effect was observed at 300 mg/kg body weight in both the extract - treated groups of mice. The
hepatoprotective effect for 100, 200 and 300 mg/kg body weight of extract was more in case of *Eclipta alba* [(ALT (45.80%, 67.09% and 84.79%), AST (52.02, 71.11 and 89.32%), ALP (21.42%, 64.29% and 89.29%) and for ACP (40.39%, 67.95% and 85.90%) activities) as compared to *Boerhavia diffusa* extract [ALT (31.31%, 48.48% and 72.46%), for AST (35.38%, 55.35% and 74.44%), for ALP (32.15%, 71.43% and 92.86%) and for ACP (31.42%, 52.57% and 72.44%) activities] in all these parameters.

The result was compared with standard drug Liv. 52 - treated group of mice (Group 13). Oral administration of Liv. 52 (300 mg/kg body weight/day) treatment along with CCl₄ significantly (p<0.05) ameliorated ALT, AST, ALP and ACP activities as compared to toxin alone treated group (Group 6). Hepatoprotective index achieved by Liv. 52 at 300 mg/kg body weight/day were 69.06% (ALT), 74.44% (AST), 85.72% (ALP) and 71.16% (ACP). The effects of both the plant extracts were higher as compared to Liv. 52 - treated group (Group 13) (Table 3.9) (Fig. 24 – 27).

**Enzymes involved in energy metabolism:**

Table 3.10 shows the effects of *Eclipta alba* and *Boerhavia diffusa* extracts on CCl₄-induced changes in energy metabolism in liver of mice. No significant changes were observed in the activities of SDH and ATPase in liver of control mice (Groups 1-5). However, a significant reduction was noted in the activities of SDH (-50.22%) and ATPase (-51.85%) in CCl₄-treated animals (Group 6) as compared to vehicle control (Group 2).

Results revealed a significant amelioration in the activities of SDH and ATPase when *Eclipta alba* *Boerhavia diffusa* extracts were concurrently administered with CCl₄ (Group 7-9, 10-12 respectively). Hepatoprotective index calculated for *Eclipta alba* was 25.59% (EA100), 64.98% (EA200) and 91.32%
(EA300) in case of SDH activity (Fig. 3.28), while 35.72% (EA100), 64.29% (EA200) and 85.72% (EA300) in case of ATPase activity (Fig. 3.29). Similarly, hepatoprotective index for SDH and ATPase activities in Boerhavia diffusa extracts - treated groups (Group 10-12) was 19.17% (BD100), 51.64% (BD200), 81.20% (BD300) (Fig. 3.28), and 28.58% (BD100), 42.86% (BD200), 64.29% (BD300) (Fig. 3.29) respectively. The effects were in a dose-dependent (EA - SDH: r = 0.9935, ATPase: r = 0.9966 and BD - SDH: r = 0.9996, ATPase: r = 0.9934) manner.

Hepatoprotective index calculated for SDH and ATPase activities were 75.23% and 57.13% respectively in Liv. 52 treated group of mice at dose of 300 mg/kg body weight (Group 13). The percentage of hepatoprotection was more for Eclipta alba as compared to Boerhavia diffusa and Liv. 52.

**Phosphorylase activity:**

Table 3.10 presents the protective effect of plant extracts on phosphorylase activity in liver of mice. No significant changes were observed in phosphorylase activity in different control groups (Group 1-5). In CCl₄ – treated group of mice (Group 6) phosphorylase activity was significantly (p<0.05) increased (170.45%) as compared to vehicle control group (Group 2).

Oral administration of Eclipta alba/Boerhavia diffusa extract at three different doses (100, 200 and 300 mg/kg body weight/day) along with CCl₄ (826 mg/kg body weight/day) significantly (p<0.05) restored phosphorylase activity in a dose-dependent manner (r = 1, 0.9971 respectively). Hepatoprotective index calculated for Eclipta alba and Boerhavia diffusa extracts treatment were 36.00% (EA100), 60.00% (EA200), 84.67% (EA300) and 34.00% (BD100), 56.67% (BD200), 74.00% (BD300) respectively (Fig. 3.30).
**Lipid peroxidation and non-enzymatic and enzymatic antioxidants:**

Table 3.11 shows the protective effects of *Eclipta alba* / *Boerhavia diffusa* extracts on CCl₄-induced changes in LPO and non-enzymatic antioxidants in liver of mice. No significant difference was noted in LPO, GSH and TAA contents between control groups of animals (Groups 1-5). However, oral administration of CCl₄ (Group 6), as compared to vehicle control (Group 2), significantly (p<0.05) reduced the GSH (-36.44%) and TAA (-55.49%) contents, while it significantly (p<0.05) increased the levels of lipid peroxidation (256.43%).

Co-administration of *Eclipta alba* / *Boerhavia diffusa* extract along with CCl₄ in three different doses (100, 200 and 300 mg/mg kg body weight/day) (Groups 7-9 and 10-12 respectively) significantly (p<0.05) decreased LPO. Protection denoted by *Eclipta alba* / *Boerhavia diffusa* extracts by CCl₄-induced LPO was 25.63% (EA100), 55.44% (EA200), 85.68% (EA300) and 20.34% (BD100), 45.41% (BD200), 77.44% (BD300) respectively as calculated by hepatoprotective index (Group 7-9 and 10-12) (Table 3.11, Fig. 3.31). The LPO levels were found to be significantly (p<0.05) decreased after oral administration of both the plant extracts along with CCl₄ in a dose-dependent manner (EA: r = 1, BD: r = 0.9975).

Non-enzymatic antioxidants (GSH and TAA) were also increased by concurrent administration of *Eclipta alba* / *Boerhavia diffusa* extracts. The effect was significant (p<0.05) and dose-dependent (EA- GSH: r = 0.9928, TAA: r = 0.9996 and BD- GSH: r = 0.9917, TAA: r = 0.9950). Hepatoprotective index calculated for GSH and TAA contents for *Eclipta alba* extracts were 43.36%, 28.36% (EA100), 68.80, 56.64 (EA200), 88.55%, 87.97% (EA300) respectively (Fig. 3.32 and 3.33 respectively). In a same manner, percent protection denoted by *Boerhavia diffusa* extracts for GSH and TAA contents were 29.83% (BD100), 57.90% (BD20), 75.68%
(BD300) and 21.96% (BD100), 42.01% (BD200), 75.64% (BD300) respectively (Fig. 3.32 and 3.33 respectively). The protective effect of both plant extracts were compared with the standard drug Liv. 52. The hepatoprotective index calculated for Liv. 52 (300 mg/kg body weight/day) - treated group (Group 13) was 74.66%, 72.76% and 44.45% for LPO, GSH and TAA contents respectively. The effects of plant extracts were higher as compared with Liv. 52 - treated group of mice (Group 13).

No significant difference in activities of CAT, SOD, GPx, GR and GST were observed between different control groups (Group 1, 2 and 3-5). Oral administration of CCl₄ (Group 6) for 30 days caused significant (p<0.05) decrease in activities of CAT (-58.98%), SOD (-59.92%), GPx (-30.65%), GR (-58.37%) and GST (-55.48%) as compared to vehicle control group (Group 2).

Enzymatic antioxidants which were significantly (p<0.05) decreased by CCl₄ treatment, which were restored back to normal by cotreatment of Eclipta alba/Boerhavia diffusa extracts in a dose-dependent manner (GSH: r = 0.9928, 9917, TAA: r = 0.9996, 0.9950 respectively). The hepatoprotective index calculated for three different doses of Eclipta alba for CAT and SOD activities were 13.72% (EA100), 43.50% (EA200), 78.68% (EA300) (Fig. 3.34) and 53.60% (EA100), 71.83% (EA200), 87.30% (EA300) (Fig. 3.35) respectively. Similarly, Hepatoprotective index calculated for GPx and GR activities were 39.48% (EA100), 76.74% (EA200), 96.06% (EA300) (Fig. 3.36) and 18.86% (EA100), 45.09% (EA200), 90.17% (EA300) (Fig. 3.37) respectively, which was 10.18% (EA100), 46.11% (EA200) and 73.66% (EA300) in case of GST activity (Table 3.12) (Fig. 3.38).

Concurrent administration of Boerhavia diffusa extract at three different doses along with CCl₄ also provides significant protection to the liver. Hepatoprotective
index for CAT and SOD activities were 6.65% (BD100), 29.64% (BD200), 60.12% (BD300) (Fig. 3.34) and 33.71% (BD100), 67.96% (BD200), 93.38% (BD300) (Fig. 3.35) respectively. In a similar manner, hepatoprotective index for GPx and GR activities were 23.62% (BD100), 52.64% (BD200), 81.58% (BD300) (Fig. 3.36) and 8.20% (BD100), 27.87% (BD200), 68.86% (BD300) (Fig. 3.37) respectively, which was 3.60% (BD100), 26.35% (BD200) and 52.70% (BD300) in case of GST activity (Fig. 3.38) (Table 3.12). Concurrent administration of CCl₄ along with Eclipta alba (Group 7-9)/ Boerhavia diffusa (Group 10-12) extracts, caused significant (p<0.05) amelioration in CCl₄-induced changes in all these parameters in a dose-dependent manner (CAT: r = 0.9988, SOD: r = 0.9764, GPx: r = 0.9992, GR: r = 0.9886, GST: r = 0.99710 in case of Eclipta alba and CAT: r = 0.9968, SOD: r = 0.9964, GPx: r = 0.9988, GR: r = 0.9800, GST: r = 0.9910 in case of Boerhavia diffusa).

Hepatoprotective index calculated for CAT, SOD, GPx, GR and GST activity was 53.74%, 81.22%, 64.48%, 59.84% and 50.30% respectively in Liv. 52 (300 mg/kg body weight) - treated group of mice (Group 13). The effects of plant extracts were higher as compared to Liv. 52 - treated group (Group 13) (Fig. 3.34 – 3.38).

**SERUM BIOCHEMICAL PARAMETERS**

*Effects on ALT, AST, ALP, ACP, LDH and γ-GT activities in serum:*

Table 3.13 represents the results of plant extract treatment on CCl₄-induced changes in the activities of liver marker enzymes in serum of mice. No significant difference in the activities of ALT, AST, ALP, ACP, LDH and γ-GT were observed among all control group animals (Groups 1 – 5). Oral administration of CCl₄ (826 mg/kg body weight/day) (Group 6) for 30 days, caused significantly (p<0.05) increased activities of all serum marker enzymes (ALT: 324.24%, AST: 342.66%,
Co-administration of *Eclipta alba*/*Boerhavia diffusa* extracts along with CCl₄, significantly (p<0.05) restored CCl₄-induced changes in all serum marker enzymes. Hepatoprotective activity of *Eclipta alba* and *Boerhavia diffusa* extract were calculated and represented as hepatoprotective index. Hepatoprotective index calculated for *Eclipta alba* extract - treated groups (Group 7-9 and 10-12) was 40.50% (EA100), 73.37% (EA200), 90.04% (EA300) in case of ALT activity (Fig. 39) and 49.57% (EA100), 80.42% (EA200), 97.15% (EA300) in case of AST activity (Fig. 40). The effect was significant and dose-dependent (ALT: r = 0.9826 and AST: r = 0.9856). In a similar manner, hepatoprotective index was 29.44%, 55.77% and 78.20% in case of ALT activity and 22.21%, 46.48% and 82.24% in case of AST activity for *Boerhavia diffusa* extract. The effect was in a dose-dependent manner (ALT: r = 0.9989 and AST: r = 0.9939).

Hepatoprotective index calculated for ALP activity was 29.27% (EA100), 51.22% (EA200), 68.30% (EA300) (Fig. 41) in case of *Eclipta alba* - treated group of mice and 26.83% (BD100), 36.59% (BD200), 68.86% (BD300) in case of *Boerhavia diffusa* extract - treated group of mice. Hepatoprotective index for ACP activity was 18.75% (EA100), 50.00% (EA200), 75% (EA300) in *Eclipta alba* - treated groups of mice (Group 7-9) and 18.75% (BD100), 50.00% (BD200), 75.00% (BD300) (Fig. 3.42) in *Boerhavia diffusa* extracts - treated groups of mice (Group 10-12). The effect was dose-dependent (ALP: r = 0.9995, 0.9608 and ACP: r = 0.9979, 0.9979) (Group 7-9 and 10-12 respectively).

Hepatoprotective index for LDH and γ-GT activities were 29.42%, 55.89%, 90.20% and 35.30%, 57.85%, 84.32% in case of *Eclipta alba* extract - treated groups.
(Groups 7-9) and 24.51%, 59.80%, 86.28% and 30.40%, 46.08%, 65.69% in case of Boerhavia diffusa extract - treated groups (Groups 10-12) at 100, 200 and 300 mg/kg body weight/day respectively (Fig. 3.43 and 3.44). The protective effect denoted by Eclipta alba Boerhavia diffusa extract was dose-dependent (EA - LDH: $r = 0.9972$, γ-GT: $r = 0.9989$ and BD – LDH: 0.9966 and γ-GT: $r = 0.9979$). Highest effect was noted at 300 mg/kg body weight/day in case of both plant extracts.

As shown in Table 3.13, Liv. 52 treatment along with CCl$_4$ caused significant reduction in all liver marker enzymes which was increased in serum of mice upon CCl$_4$ alone treatment. The protection denoted by Liv. 52 at 300 mg/kg body weight dose for ALT, AST, ALP, ACP, LDH and γ-GT activities were 72.43%, 79.15%, 70.74%, 62.50%, 81.38% and 62.75% respectively. Protective effect of both the plant extracts was higher as compared to Liv. 52 - treated group of mice (Group 13) (Fig. 3.39 - 3.44).

**Effects on bilirubin contents in serum:**

Table 3.14 represents the hepatoprotective effect of Eclipta alba and Boerhavia diffusa extracts on serum bilirubin contents. Oral administration of CCl$_4$ had resulted in increased contents of total bilirubin (150.36%), direct bilirubin (103.75%) and indirect bilirubin (213.56%) significantly (p<0.05) as compared to vehicle control group (Group 2).

Concurrent administration of Eclipta alba extract along with CCl$_4$ provides significant (p<0.05) protection to the liver. Hepatoprotective index calculated for total bilirubin [13.40% (EA100), 53.59% (EA200), 78.95% (EA300) (Fig. 3.34)], direct bilirubin [27.11% (EA100), 53.02% (EA200), 79.52% (EA300) (Fig. 3.35)] and for indirect bilirubin [42.07% (EA100), 54.77% (EA200), 82.54% (EA300) (Fig. 3.36)] content was dose-dependent (TB: $r = 0.9920$, DB: $r = 0.9999$, IB: $r = 0.9776$) in
Eclipta alba - treated groups of mice (Group 7-9). In a similar way, hepatoprotective index was calculated for Boerhavia diffusa - treated groups of mice was 13.40%, 53.59% and 78.95% in case of total bilirubin content (Fig. 3.45), 12.05%, 32.54% and 68.68% in case of direct bilirubin content (Fig. 3.46) and 14.29%, 67.47% and 86.51% in case of indirect bilirubin content (Fig. 3.47) at doses of 100, 200 and 300 mg/kg body weight respectively. The effect was dose-dependent (TB: r = 0.9916, DB: r = 0.9875, IB: r = 0.9756). In Liv. 52 - treated group (Group 13), hepatoprotective index was 72.73%, 66.27% and 76.99% for total bilirubin, direct bilirubin and indirect bilirubin contents at a dose of 300 mg/kg body weight/day respectively. Protection provided in plant extracts treated groups were compared with Liv. 52 - treated group and the protective effect of both the plant extracts were higher as compared to Liv. 52 - treated group (Group 13).

Effects on serum protein, albumin, cholesterol as well as blood glucose contents:

Table 3.15 represents the protective effects of plant extracts on biochemical parameters in serum and blood of mice. No significant alteration was observed among untreated control, vehicle control and antidote control groups in protein, albumin, cholesterol in serum and blood glucose contents (Group 1, 2 and 3-5). However, oral administration of CCl₄ (826 mg/kg body weight/day, Group 6) caused significant (p<0.05) decrease in protein (52.84%) and albumin (64.54%) contents, while significant (p<0.05) increase was observed in blood glucose (105.54%) and cholesterol (87.21%) contents.

Concurrent administration of Eclipta alba/Boerhavia diffusa extract along with CCl₄ (Group 7-9 and 10-12) caused significant (p<0.05) increase in serum protein and albumin contents, while its administration significantly decreased the
blood glucose and serum cholesterol contents. The hepatoprotective index was 48.36%, 62.99% and 81.80% for serum protein content (Fig. 3.48), 32.09%, 57.34% and 84.65% for serum albumin content (Fig. 3.49), 27.06%, 65.90% and 91.01% for blood glucose content (Fig. 3.50) and 40.00%, 76.00%, 93.34% for serum cholesterol content (Fig. 3.51) in case of *Eclipta alba* - treated groups (Groups 7-9). In similar manner, hepatoprotective index for serum protein content 30.45%, 45.68% and 60.30% (Fig. 3.48), for serum albumin content 21.85%, 47.45% and 72.02% (Fig. 3.49), for serum cholesterol contents 32.00%, 49.34% and 81.34% (Fig. 3.50) and for blood glucose content 12.62%, 50.70% and 79.93% (Fig. 3.51) and at 100, 200 and 300 mg/kg body weight dosage respectively (Groups 10–12). The hepatoprotective effects of both the plant extracts were in dose-dependent manner (EA-r = Protein: 0.9974, Albumin: 0.9997, Glucose = 0.9924, Cholesterol: 0.9802 / BD-r = Protein: 0.9999, Albumin: 0.9999, Glucose: 0.9971, Cholesterol: 0.9856).

Oral administration of Liv. 52 also provides significant (p<0.05) protection in all these parameters. The hepatoprotective index indicated that the protective effect of *Eclipta alba* was highest, followed by *Boerhavia diffusa* and Liv. 52.

**Correlation analysis:**

Tables 3.16 and 3.17 represents the Pearson correlation analysis among the liver marker enzymes (AST, ALT, ACP, ALP, LDH, γ-GT, TB, DB and IB) in serum and lipid peroxidation (LPO) in liver of mice. It has been found that there is significant positive correlation among all parameters in serum and lipid peroxidation in liver with correlation coefficient (r) in the range of 0.9217-1.0000 in *Eclipta alba* - treated groups (Table 3.16) and 0.8539-1.0000 in *Boerhavia diffusa* - treated groups (Table 3.17) of mice.
Tables 3.18 and 3.19 represent the Pearson correlation analysis between LPO content in liver and liver antioxidative parameters CAT, SOD, GPx, GR, GST, GSH and TAA in liver of mice. The results indicate that there is significant positive correlation among all the antioxidative parameters with correlation (r) values in the ranges of 0.9430-1.0000 and 0.9463-0.9999 in *Eclipta alba* (Table 3.18) and *Boerhavia diffusa* (3.19) extracts respectively.