

# Pharmacological Potential of *Chrozophora Tinctoria* Extracts for the Management of Carbon Tetra Chloride (Ccl<sub>4</sub>) Induced Hepatotoxicity in Rat's Model

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## Abstract

**Background:** Liver is a vital organ of the human body, and hepatotoxicity is major problem of liver and the current scenario is effected in this problem in a big ratio it has many causes, in this study the hepatotoxicity induced in rat to use CCl<sub>4</sub> and its treated by hydro-alcoholic extract of *C.tinctoria* to given low and high dose and was to be evaluated by the comparison between disease and treatment group.

**Objective:** The present study was design to carry out for the evaluation of hepatoprotective effects of hydro alcoholic leaves extract of *Chrozophora tinctoria* over the carbon tetrachloride (CCl<sub>4</sub>) induced liver damage in albino Wister rats.

**Methods:** All the experimental Wister rats were randomly divided into five groups having six rats in each. Group I served as the normal control (Saline 0.9%) rat, Group II, Group III, Group IV and Group V was administered with CCl<sub>4</sub> which was prepared in 20% olive oil at a signal dose of 1.5ml/kg body weight by i.p. route for induction of hepatotoxicity. In an experimental protocol Group II serves as diseased control. Group III: was administered with hydro alcoholic extract of *C.tinctoria* (50mg/kg/day), Group IV: was treated with hydro alcoholic extract of *C.tinctoria* (100mg/kg/day). Group V: was treated with sylimarin (25mg/kg /day). Over the experimental period of 28 days blood sample were collected for biochemical analysis, and liver tissue were collected for biopsy.

**Result:** After administration of CCl<sub>4</sub> rats developed hepatotoxicity which evident by elevation in AST, ALT, ALP, total bilirubin, total proteins and the antioxidant enzyme parameters CAT, SOD, GGT, GST was decreases. Although administer of CCl<sub>4</sub>. Administration of hydro alcoholic extract of *C.tinctoria* (50mg/kg /day) shows improved in AST, ALT, ALP, total bilirubin, total proteins towards normal level and significantly increases (\*\*p<0.05) the antioxidant enzyme parameters CAT, SOD, GGT, and GST. Similarly, administration of hydro alcoholic extract of *C.tinctoria*(100mg/kg/day) shows more significantly decrease (\*\*p<0.05) in AST, ALT, ALP, total bilirubin, total protein towards normal level increases and more significantly (\*\*p<0.05)the antioxidant enzyme parameters CAT, SOD, GGT, and GST in compare to standard as well as control groups. However, the albino Wister rats treated with high dose hydro alcoholic extract (*C.tinctoria* 100mg) showed more significant improvements as hepatoprotective than low dose of *C.tinctoria* i.e.50mg.

**Discussion:** The hepatoprotective activity of hydro alcoholic extract of *C.tinctoria* 100mg could palliate the liver injuries perhaps by its phytoconstituents and antioxidant enzyme, hence it eliminating the deleterious effect of toxic metabolites from the CCl<sub>4</sub>.

**Keywords:** Chrozophora Tinctoria, Carbon Tetra Chloride, Hepatoprotective, AST, ALT, ALP.

## Introduction

Hepatotoxicity is kind of liver dysfunction or damage liver which is associated with improper uses of antibiotics and potent drugs.<sup>[1]</sup> Those chemicals which cause liver injury are called hepatotoxins or hepatotoxic agents.<sup>[2]</sup> Hepatotoxicity is a phenomenon which implies as chemical agents drive liver damage. Drug induced liver injuries is a major cause of acute and chronic liver diseases.<sup>[3]</sup> The liver plays a central role in transforming and clearing the foods and chemicals to minimize the susceptible of these agents as toxic.<sup>[4]</sup> Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, and natural chemicals like microcystins, herbal remedies and dietary supplements. Certain drugs may cause liver injuries when they introduced even within the minimum safety concentration ranges.<sup>[5]</sup> Most of the time reactive metabolites and immune mediated agents are more prone towards the cause hepatotoxicity rather than primary compounds for affecting hepatocytes, biliary epithelial cells and or liver vasculature.<sup>[6]</sup> **Chemicals causing of Hepatotoxicity:** Carbon tetrachloride (CCl<sub>4</sub>), Thioacetamide (TTA), Diethyl nitrosamine (DEN),<sup>[7]</sup> Aflatoxin B1 (AFB1), Bromobenzene, Lithotomic acid, Acryl amide (AA), Acrolein (allyl alcohol), Alpha-Naphthyl Isothiocyanate (ANIT).<sup>[8]</sup> The prepared extract of *Chrozophora tinctoria* is an annual plant belonging to family *Euphorbiaceae* and exclusively described as a species in 1824 and comprises monoecious herbs or under shrubs.<sup>[9]</sup> The species of this plant is well known across Europe, Africa, and Asia. *Chrozophora tinctoria* is a yearly level to the ground herb widespread of dry waste spaces on sandy clay and the flowering condition observed in the months of April to June. It can grow up in semi shade (light inhabitant to Africa, tropical Asia, and Europe).<sup>[10]</sup> The plant is used to care for warts, emetic, cathartic, and fever whereas root ashes are given to children for cough. The seeds are purgative or cathartic, even though its bark is used for tanning and coloring. Various useful chemical constituents naturally present in the leaves playing an important role in the field of pharmacological behavior on living system. It has also possess wound healing properties<sup>[11]</sup> *Chrozophora tinctoria* which are identified as, (1) amentoflavone, (2) apigenin-7-O-β-D-glucopyranoside, (3) apigenin-7-O-6''-E-pcoumaroyl-β-D-glucopyranoside, (4) acacetin-7-O-β-D-[α-L-rhamnosyl(1→6)]3''-E-p-coumaroyl glucopyranoside, (5) apigenin7-O-(6''-Z-p-coumaroyl)-β-D-glucopyranoside, and (6) rutin. This study was designed to evaluate the protective effect of *Chrozophora tinctoria* against CCl<sub>4</sub>induced hepatotoxicity in rats.<sup>[12]</sup> The extent of the protective effect was analyzed by studying serum and toxic markers and confirm with histopathology of Liver tissues.<sup>[13]</sup>



Figure 1: Hepatotoxicity induced by single dose administration of ccl<sup>4</sup>

## Materials and Method

### Plant Collection and Authentication

The fresh herbal plant *C. tinctoria* is collected in the months of May-June from the district of Ambedkar Nagar, Uttar Pradesh (India). Fresh and shade dried leaves have been collected consequently and authenticated by Botanical Survey of India, Northern regional center 192, Kaulagarh road, Dehradun 248195, with plant Acc. number 114546.

### Preparation of Extract

*Chrozophora tinctoria* was prepared by hydro-alcoholic leaf extract of plants (200gm) by using cold maceration method. The collected extract was dried by using rotatory evaporator at temperature of  $45^{\circ}\text{C} \pm 5^{\circ}\text{C}$  until the entire solvent get evaporated. The obtained dried extract powder was stored in a well tightly closed container.<sup>[14]</sup>

### Experimental Animals

Healthy adult Albino Wister rats (3-4 months old) with body weight 150-200gm of either sex were selected for the study. The animals were kept in well-ventilated animal house under natural conditions with 12 hours in light and dark cycle at  $25 \pm 2^{\circ}\text{C}$  room temperature and followed by acclimatization for a period of 2 weeks by maintaining standard environmental conditions and fed with standard pellet diet & water. The experiments were carried out in accordance with the CPCSEA guidelines and Institutional animal ethical clearance. The IAEC approved protocol no. HIPER/IAEC/18/18/04. Animals were randomized into five groups with 6 animals in each group.

### Experimental Design

After acclimatization all Wister rats (150-200g) were divided into five groups having six animals in each. The study was design and conducted for 28 days. Group I treated as normal control(administered vehicle only) for 28 days. Group II Disease control group (Induced  $\text{CCl}_4$  1.5ml/kg/i.p.), continued with vehicle. *Therapeutic groups:* Each groups were first induced hepatotoxicity with  $\text{CCl}_4$  1.5ml/kg/b.w for 28 days of study. After inducing each group with  $\text{CCl}_4$ , every group were divided into three different treatments. Group III therapeutic groups treated with Hydro-alcoholic Extract of *C.tinctoria* at low dose (50mg/kg/b.w. p.o.). Group IV therapeutic groups treated with Hydro-alcoholic Extract of *C.tinctoria* at high dose (100mg/kg/b.w. p.o.). Group V Silymarin as standard (25mg/kg b.w. p.o.) for 28 days. All the experiment continued for 28 days.

## Biochemical Estimations

### Body Weight

Animals were weighed at the start of experiment and their final body weight using an electronic balance at the end of experiment.

### LFT Profile in Blood

It include various parameters and its normal rang if any changes in its normal value that means some abnormality in the liver Blood bilirubin, Total protein, blood ammonia, AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase),ALP (Alkaline Phosphatase), albumin in serum, globulins in blood, and serum prothrombin time <sup>[15]</sup>

### Antioxidant Activity

The liver tissue of each rat were removed immediately, washed in saline, blotted between filter paper fold to dryness and weighed. Then the liver was homogenized in phosphate buffer (pH – 7.4) to give a 10% homogenate. Antioxidant properties like, Super oxidase (SOD), Catalase (CAT), Gamma-glutamyl transferase (GGT) and Glutathione-S-transferase (GST) were performed. <sup>[16]</sup>

### Histopathological Analysis

Liver tissues were obtained at the end of study after scarification of animals and immediately fixed in 10% neutral formalin buffered solution. The tissue section was stained with hematoxylin and eosin (H & E stain) and examined under the light microscope at 40x. <sup>[17]</sup>

**Processing of isolated liver:** The animals were sacrificed and the liver of each animal were isolated and cut into small pieces, preserved and fixed in 10% formalin for two days. Then the liver pieces were washed in running water for about 12 hours to remove the formalin and were followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with three changes for 12 hours each. After paraffin infiltration the liver pieces were subjected to automatic tissue processing unit. Embedding in paraffin vacuum hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allowed to cool. <sup>[18]</sup>

**Biopsy of liver tissue:** The blocks were cut using microtome to get sections of thickness of 5 $\mu$ . The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in an oven at 60°C for 1 hour. Paraffin melts and egg albumin denatures, there by fixing of tissue to slide. <sup>[19]</sup>

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

### Statistical Analysis

All the data were expressed as Mean $\pm$ SEM followed by one way ANOVA with Bonferroni's multiple comparison test (n=6). #P<0.05 represent statistically significance against normal control, while \*\*P<0.05 represent statistically significance against disease control; whereas NS show non significance comparison between the groups.

## Results and Discussion

### Phytochemical investigation

In order to identify chemical constituents of the extracts preliminary chemical tests were performed. Preliminary qualitative tests were carried out to assess presence or absence of various phytoconstituents like phenols, amino acids, proteins, fatty acids, carbohydrates, alkaloids, sterols, Flavonoids, glycosides, and saponins. <sup>[20]</sup>

**Table 1: Phytochemical investigation of extracts**

| S.No. | Phytoconstituents | Name of the test     | Result |
|-------|-------------------|----------------------|--------|
| 1.    | Phenols           | Ferric chloride test | +++    |
| 2.    | Amino acids       | Biuret test          | +++    |
| 3.    | Proteins          | Biuret test          | +      |
| 4.    | Fatty acids       | Spot test            | ++     |
| 5.    | Carbohydrates     | Molischs test        | +      |
| 6.    | Alkaloids         | Dragendorff's test   | ++     |

|     |            |                           |     |
|-----|------------|---------------------------|-----|
| 7.  | Sterols    | Liebermann-Burchards test | +++ |
| 8.  | Flavonoids | Shinoda test              | +++ |
| 9.  | Glycosides | Legal's test              | +   |
| 10. | Saponins   | Foam test                 | ++  |

+++ : highly present; ++ moderately present; + slightly present; –absent.

It was observed that extracts of *C.tinctoria* leaves showed presence of phenols, amino acids, proteins, fatty acids, carbohydrates, alkaloids, sterols, flavonoids, glycosides, and saponins. Presence of phenol it is useful in relieve pain and useful in case of cancer, flavonoids are powerful antioxidants and anti-inflammatory and immune system benefits, sterol are shows anti-inflammatory activity there are several uses of these phytochemicals constituents.

### Biochemical analysis of serum samples

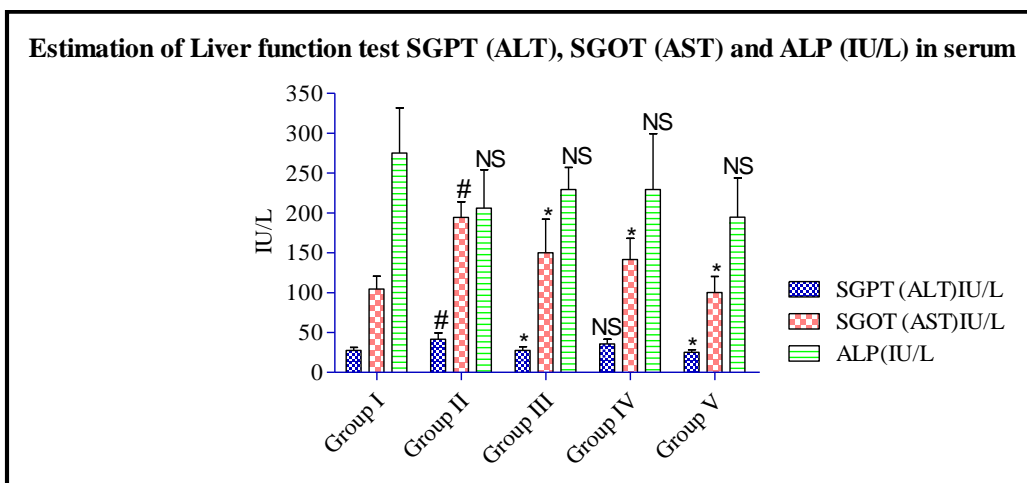
Serum samples collected from different groups were analyzed for the evaluation of the level Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphate (ALP). Evaluation of ALT, AST and ALP with different experiment groups which are described in the table no. 2. [21, 22]

**Table 2: Estimation of Liver function test SGPT (ALT) IU/L, SGOT (AST) IU/L and ALP (IU/L) in serum of experimental rats.**

| Group Description                               | ALT (IU/L)                 | AST (IU/L)                   | ALP(IU/L)                    |
|-------------------------------------------------|----------------------------|------------------------------|------------------------------|
| Normal control (Saline 0.9%)                    | 27.500±3.860               | 104.670±15.860               | 275.170±56.600               |
| Disease control (CCl <sub>4</sub> 1.5ml/kg)     | 41.670±7.739 <sup>#</sup>  | 194.500±19.397 <sup>#</sup>  | 206.000±48.294 <sup>#</sup>  |
| CCl <sub>4</sub> + <i>C. tinctoria</i> 50mg/kg  | 27.500±4.787 <sup>**</sup> | 150.170±41.950 <sup>**</sup> | 229.500±27.548 <sup>**</sup> |
| CCl <sub>4</sub> + <i>C. tinctoria</i> 100mg/kg | 35.670±5.910 <sup>**</sup> | 141.50±26.690 <sup>**</sup>  | 247.500±70.040 <sup>**</sup> |
| CCl <sub>4</sub> + Silymarin 25mg/kg            | 25.170±3.130 <sup>**</sup> | 100.33±19.920 <sup>**</sup>  | 194.670±49.180 <sup>**</sup> |

First group of the experiment was normal control group (Saline 0.9%) this group shows the value of ALT (27.500±3.860)IU/L, AST (104.670±15.860) IU/L, ALP (275.170±56.600) IU/L this is the normal values, second group was disease control group which is induced hepatotoxicity with CCl<sub>4</sub> (CCl<sub>4</sub>1.5ml/kg) from Table: (2) if we see the values of ALT (41.670±7.739) IU/L, AST (194.500±19.397) IU/L and ALP (206.000±48.294) IU/L of disease control group we found increasing level of biomarkers which indicates the disease condition. The third group of this experiment is CCl<sub>4</sub>+ *C. tinctoria* 50mg/kg this was the low dose of *C. tinctoria* and the values of ALT (27.500±4.787) IU/L, AST (150.170±41.950) IU/L and ALP (229.500±27.548) IU/L of this group was increases and shows approximately similar range in comparison to normal control group and standard control group, the fourth group of this experiment was CCl<sub>4</sub> + *C. tinctoria* 100mg/kg this group was show the approximately similar range of ALT (35.670±5.910) IU/L, AST (141.50±26.690) IU/L and ALP (247.500±70.040) IU/L in comparison to standard and normal control group, the fifth group CCl<sub>4</sub> + Silymarin 25mg/kg of this experiment was show the approximately slandered range of ALT (25.170±3.130) IU/L, AST (100.33±19.920) IU/L, ALP (194.670±49.180) IU/L.

Graph 1: Estimation of Liver function test SGPT (ALT) IU/L, SGOT (AST) IU/L and ALP (IU/L)



Values are given as Mean  $\pm$  SEM of experimental animals (n=6); NS=Non significance

#P<0.05 represents statistical significance against normal control,

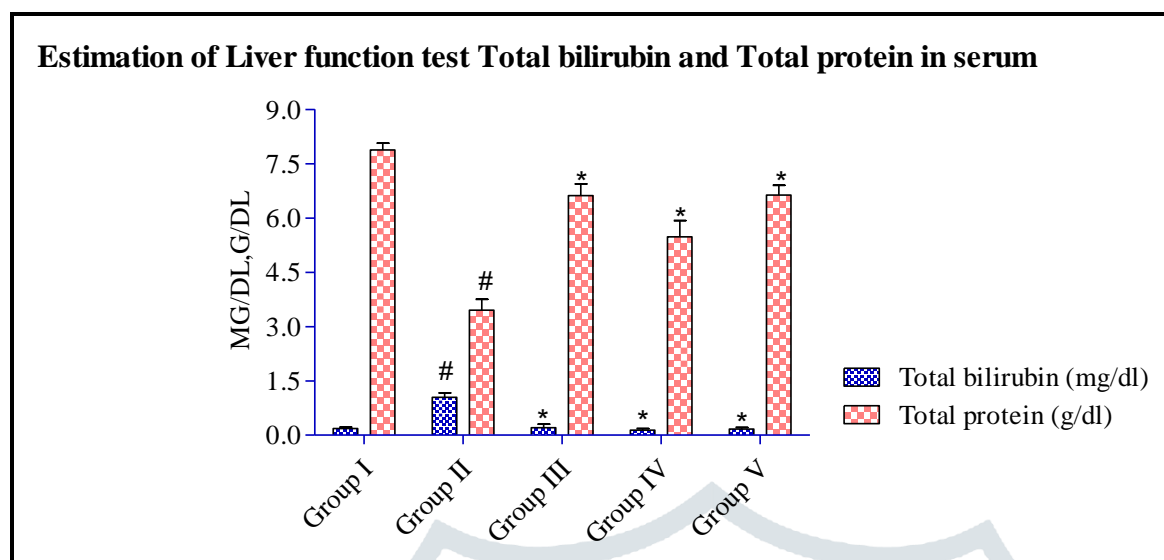
\*\*P<0.05 represents statistical significance against disease control

**Table 3: Estimation of Liver function test as Total bilirubin (mg/dl) and Total protein (g/dl) in serum of experimental rats**

| Group No. | Group Description                               | Total bilirubin (mg/dl)         | Total protein (g/dl)            |
|-----------|-------------------------------------------------|---------------------------------|---------------------------------|
| I         | Normal control (Saline 0.9%)                    | 0.180 $\pm$ 0.040               | 7.880 $\pm$ 0.190               |
| II        | Disease control (CCl <sub>4</sub> 1.5ml/kg)     | 1.050 $\pm$ 0.115 <sup>#</sup>  | 3.450 $\pm$ 0.305 <sup>#</sup>  |
| III       | CCl <sub>4</sub> + <i>C. tinctoria</i> 50mg/kg  | 0.210 $\pm$ 0.092 <sup>**</sup> | 6.620 $\pm$ 0.322 <sup>**</sup> |
| IV        | CCl <sub>4</sub> + <i>C. tinctoria</i> 100mg/kg | 0.140 $\pm$ 0.040 <sup>**</sup> | 5.480 $\pm$ 0.450 <sup>**</sup> |
| V         | CCl <sub>4</sub> + Silymarin 25mg/kg            | 0.170 $\pm$ 0.040 <sup>**</sup> | 6.630 $\pm$ 0.270 <sup>**</sup> |

The evaluation of total bilirubin and total protein reveals that first group was shows the normal range of total bilirubin (0.180 $\pm$ 0.040) and total protein (7.880 $\pm$ 0.190), second group (CCl<sub>4</sub> 1.5ml/kg) the range of total bilirubin (0.180 $\pm$ 0.040) was decreases and total protein (3.450 $\pm$ 0.305) increases that indicate the disease condition, group third was CCl<sub>4</sub>+ *C.tinctoria* 50mg/kg the result value of this group indicates that total bilirubin (0.210 $\pm$ 0.092) and total protein (6.620 $\pm$ 0.322) was nearby approximate in compare to normal control group, the fourth group was CCl<sub>4</sub> + *C. tinctoria* 100mg/kg that was shows the total bilirubin (0.140 $\pm$ 0.040) and total protein (5.480 $\pm$ 0.450) indicated the range approximately similar to control and standard group, the fifth group was standard CCl<sub>4</sub> + Silymarin 25mg/kg group the value of this group shows nearby approximately range of total bilirubin (0.170 $\pm$ 0.040) and total protein (6.630 $\pm$ 0.270).

Graph 2: Estimation of Liver function test as Total bilirubin (mg/dl) and Total protein (g/dl)



Values are given as

Mean  $\pm$  SEM of experimental animals (n=6); NS=Non significance #P<0.05 represents statistical significance against normal control,

\*\*P<0.05 represents statistical significance against disease control.

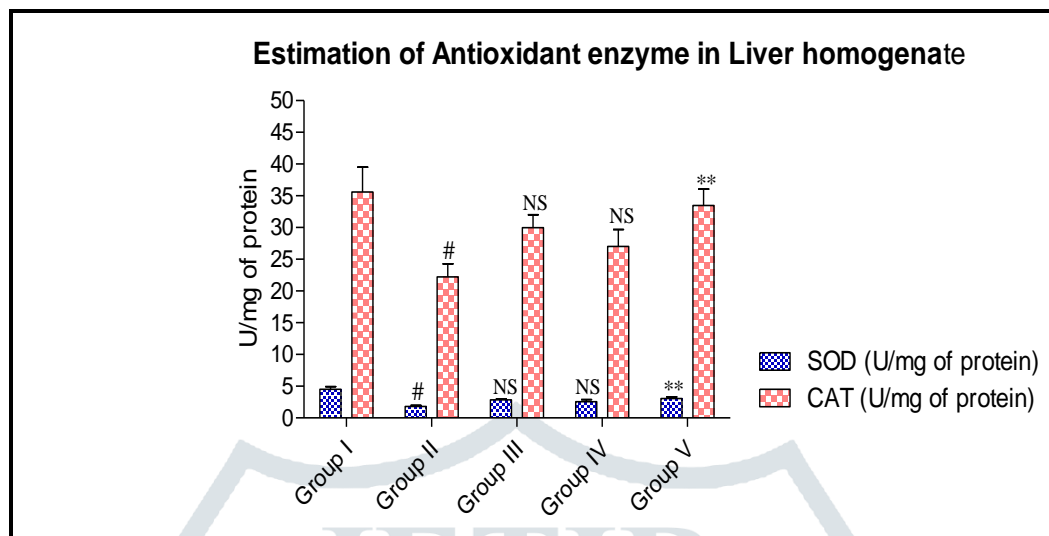
**Table 4: Estimation of Antioxidant enzyme SOD (U/mg of protein) and CAT (U/mg of protein) in Liver homogenate**

| Group No. | Group Description                           | SOD (U/mg of protein)           | CAT (U/mg of protein)            |
|-----------|---------------------------------------------|---------------------------------|----------------------------------|
| I         | Normal control (Saline 0.9%)                | 4.520 $\pm$ 0.400               | 35.620 $\pm$ 3.920               |
| II        | Disease control (CCl <sub>4</sub> 1.5ml/kg) | 1.830 $\pm$ 0.130 <sup>#</sup>  | 22.240 $\pm$ 2.010 <sup>#</sup>  |
| III       | CCl <sub>4</sub> + C. tinctoria 50mg/kg     | 2.860 $\pm$ 0.150 <sup>NS</sup> | 29.990 $\pm$ 2.010 <sup>NS</sup> |
| IV        | CCl <sub>4</sub> + C. tinctoria 100mg/kg    | 2.543 $\pm$ 0.352 <sup>NS</sup> | 27.010 $\pm$ 2.689 <sup>NS</sup> |
| V         | CCl <sub>4</sub> + Silymarin 25mg/kg        | 3.070 $\pm$ 0.230 <sup>**</sup> | 33.480 $\pm$ 2.580 <sup>**</sup> |

Estimation of antioxidant enzyme SOD (U/mg of protein) and CAT (U/mg of protein) in Liver homogenate shows in Table:4. The value of group first was shows the normal range of SOD (4.520 $\pm$ 0.400) U/mg and normal range of CAT (35.620 $\pm$ 3.920), the disease control group(CCl<sub>4</sub> 1.5ml/kg) show the value of SOD (1.830 $\pm$ 0.130) and the value of CAT (22.240 $\pm$ 2.010) was decreases in this group (1.830 $\pm$ 0.130) and the value of CAT, the third group was CCl<sub>4</sub>+ C. tinctoria 50mg/kg which shows that value of SOD (2.860 $\pm$ 0.150) and the value of CAT (29.990 $\pm$ 2.010) was decreases in compare to control group, the fourth group i.e. CCl<sub>4</sub> + C. tinctoria 100mg/kg was shows the value of SOD (2.543 $\pm$ 0.352) and the value of CAT (27.010 $\pm$ 2.689) this value significantly decreases, compare to control group and increases in comparison to disease control group, the fifth group of this table was standard group CCl<sub>4</sub> + Silymarin

25mg/kg this group was shows the SOD ( $3.070\pm 0.230$ ) and value of CAT was ( $33.480\pm 2.580$ ) this range was indicate the approximately similar range in compare to normal control group.

Graph 3: Estimation of Antioxidant enzyme SOD (U/mg of protein) and CAT (U/mg of protein)



Values are given as Mean  $\pm$  SEM of experimental animals (n=6); NS=Non significance #P<0.05 represents statistical significance against normal control,

\*\*P<0.05 represents statistical significance against disease control.

Table 5: Estimation of Antioxidant enzyme GGT (nmol/min) and GST (nmol/min) in Liver homogenate

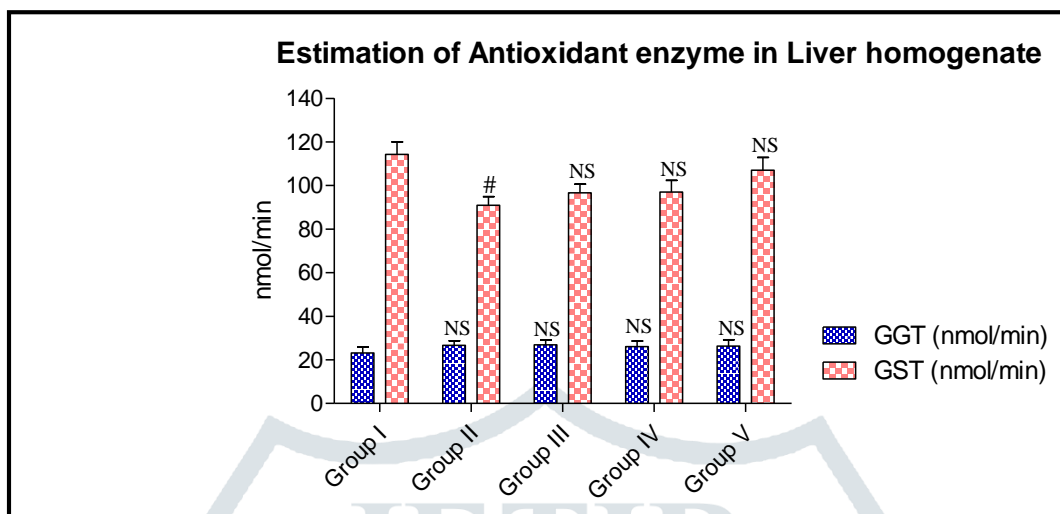
| Group No. | Group Description                           | GGT (nmol/min)                   | GST (nmol/min)                    |
|-----------|---------------------------------------------|----------------------------------|-----------------------------------|
| I         | Normal control (Saline 0.9%)                | 23.160 $\pm$ 2.700               | 114.260 $\pm$ 5.690               |
| II        | Disease control (CCl <sub>4</sub> 1.5ml/kg) | 26.710 $\pm$ 2.000 <sup>NS</sup> | 90.920 $\pm$ 4.020 <sup>#</sup>   |
| III       | CCl <sub>4</sub> + C. tinctoria 50mg/kg     | 26.850 $\pm$ 2.260 <sup>NS</sup> | 96.680 $\pm$ 4.000 <sup>NS</sup>  |
| IV        | CCl <sub>4</sub> + C. tinctoria 100mg/kg    | 26.042 $\pm$ 2.655 <sup>NS</sup> | 96.967 $\pm$ 5.357 <sup>NS</sup>  |
| V         | CCl <sub>4</sub> + Silymarin 25mg/kg        | 26.270 $\pm$ 2.910 <sup>NS</sup> | 107.050 $\pm$ 5.860 <sup>NS</sup> |

Estimation of antioxidant enzyme GGT (nmol/min) and GST (nmol/min) in liver homogenate was shows in Table:5. The value of first group was shows the normal range of GGT ( $23.160\pm 2.700$ ) nmol/min and normal range of GST ( $114.260\pm 5.690$ ) nmol/min, the second group of this study was disease control group (CCl<sub>4</sub> 1.5ml/kg) the value of GGT ( $26.710\pm 2.000$ ) nmol/min increases and the value of GST ( $90.920\pm 4.020$ ) nmol/min decreases this was indicated the disease condition, the third group of this table was CCl<sub>4</sub> + C. tinctoria 50mg/kg the value of GGT ( $26.850\pm 2.260$ ) nmol/min increases and the value of GST ( $96.680\pm 4.000$ ) nmol/min decreases in compare to normal control group, the fourth group was CCl<sub>4</sub> + C. tinctoria 100mg/kg this group was shows the value of GGT ( $26.042\pm 2.655$ ) nmol/min this value was increases and the value of GST was ( $96.967\pm 5.357$ ) nmol/min this value was decreases in compare to normal control group, the fifth group of this table was standard group CCl<sub>4</sub> + Silymarin 25mg/kg this group was shows the



GGT ( $26.270 \pm 2.910$ ) nmol/min and value of GST was ( $107.050 \pm 5.860$ ) nmol/min this range was indicate the approximately similar range in compare to normal control group.

Graph 4: Estimation of Antioxidant enzyme GGT (nmol/min) and GST (nmol/min)



Values are given as Mean  $\pm$  SEM of experimental animals (n=6); NS=Non significance

#P<0.05 represents statistical significance against normal control,

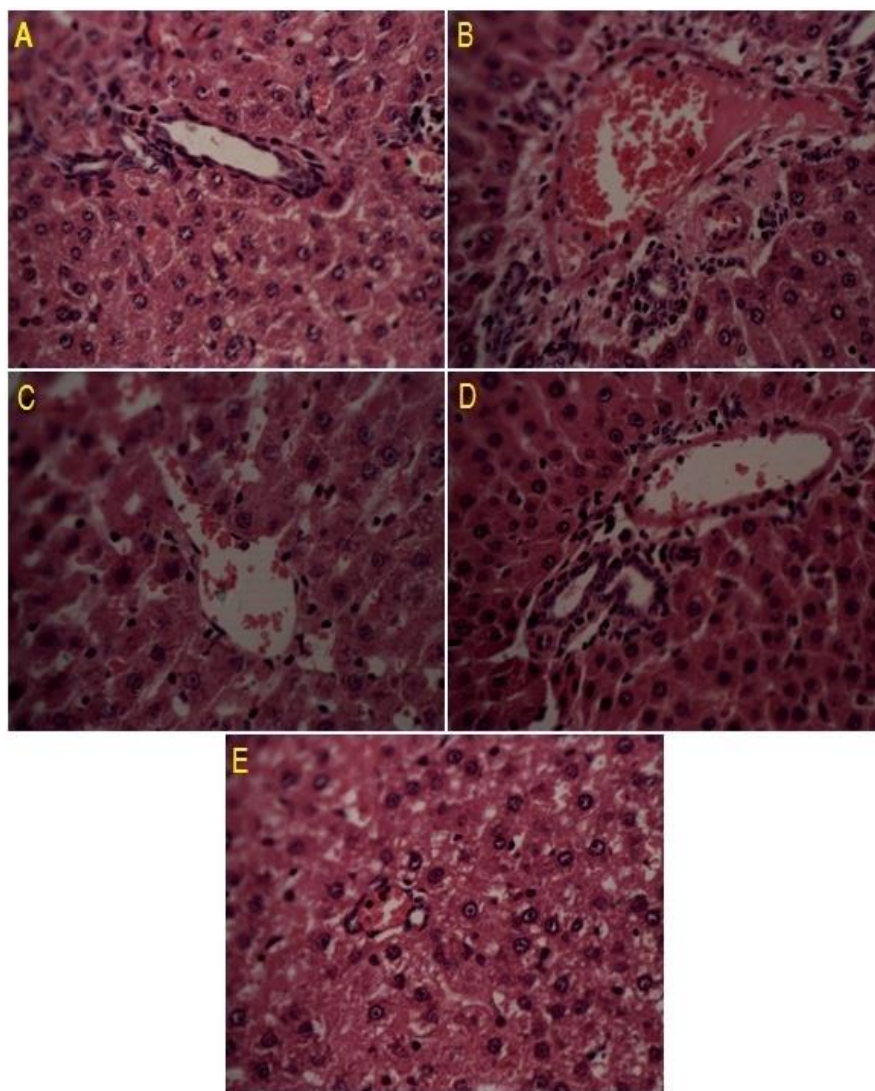
\*\*P<0.05 represents statistical significance against disease control.

### Body Weight

The average body weight of animal in CCl<sub>4</sub> treated rat showed a slight decrease in body weight (174g) compared with treatment as well as normal control rat (194g), while the treatment with *C.tinctoria* and standard drug treated animals showed a little increased in body weight (186g) as compared to CCl<sub>4</sub> treated group of animal.

### Histopathological parameters

Liver tissues were obtained on last day of the experiment after scarification of animals and immediately fixed in 10% buffered neutral formalin solution. The tissue section was stained with hematoxylin and eosin (H & E stain) and examined under light microscope. The histological changes such as fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis, presence of fatty cyst and interstitial inflammation, etc. were observed in all group of the rat (figure 2).



**Figure 2:** Section of the albino Wister rat liver shows in different experiment group (A) Normal control (Saline 0.9%), (B) Disease control ( $CCl_4$  1.5ml/kg), (C)  $CCl_4$ + *C. tinctoria* 50mg/kg, (D)  $CCl_4$  + *C. tinctoria* 100mg/kg, (E)  $CCl_4$  + Silymarin 25mg/kg.

Histopathological profile of the liver tissues shows in control group normal hepatic cells each with well-preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein Figure 2(A).  $CCl_4$  intoxicated rats has shown the fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis and presence of fatty cyst. The sinusoids of liver were congested and the central vein of globule was constricted Figure 2(B). The animal treated with hydro-alcoholic extract of *C. tinctoria* low dose (50mg/kg) exhibited significant liver protection against the toxic substance as evident by normal lobular pattern with a mild degree of fatty change, absence of necrosis and lymphocyte infiltration mild comparable to the standard and control Figure 2(C). The animal treated with hydro-alcoholic extract of *C. tinctoria* high dose (100mg/kg) showed the liver architecture more effective than low dose and almost comparable to the standard and control. However, accumulation of fatty lobules (steatosis), necrosis and scattered lymph mononuclear (LMN) cell infiltrate in hepatic parenchyma were noticed in the section of animal treated with hydro-alcoholic extract of *C. tinctoria* Figure 2(D). The section of liver was taken from the animals treated with standard drug silymarin (25mg/kg) showed the hepatic architecture, which was similar to that of control Figure 2(E).

## Conclusion

The present study is directed towards investigate the hepatoprotective activity of plant *C.tinctoria* by using hydro-alcoholic extract. Many hepatoprotective plants are available but scientific validation and standardization is very difficult for available plant extracts. In present study, hydro-alcoholic extract of *C.tinctoria* was study on albino Wister rat and well-tested pathological, histopathological and morphological parameters on laboratory basis were observed. This experimental study was concluded on the basis of phytochemical investigation, biochemical analysis of serum sample, antioxidant parameters, morphological parameters, and histopathological study of liver tissue. Phytochemical investigation shows the presence of phenols, amino acids, proteins, fatty acids, carbohydrates, alkaloids, sterols, flavonoids, glycosides, and saponins, presence of phenol it is useful in relieve pain and useful in case of cancer, flavonoids are powerful antioxidants and anti-inflammatory and immune system benefits, sterol are shows anti-inflammatory activity on the basis of study of these chemical constituent it may be also useful for hepatoprotective activity. Biochemical analysis like Aspartate Transaminase (AST) ( $141.50\pm 26.690$ ), Alanine Transaminase (ALT) ( $35.670\pm 5.910$ ) Alkaline Phosphate (ALP) ( $247.500\pm 70.040$ ), total bilirubin ( $0.140\pm 0.040$ ), and total protein ( $5.480\pm 0.450$ ) on the basis of observation of these parameter shows significant value in these group  $CCl_4 + C. tinctoria$  50mg/kg,  $CCl_4 + C. tinctoria$  100mg/kg,  $CCl_4 + Silymarin$  25mg/kg groups in compare to normal control group, group the fourth group  $CCl_4 + C. tinctoria$  100mg/kg,  $CCl_4$  was shows more significant value in compare to standard and control group so high dose of hydro-alcoholic extract of *C.tinctoria* was more effective in compare to low dose. Antioxidant enzyme parameters like super oxidase (SOD) ( $2.543\pm 0.352$ ), catalase (CAT) ( $27.010\pm 2.689$ ), gamma-glutamyl transferase (GGT) ( $26.042\pm 2.655$ ), glutathione-S-transferase GST ( $96.967\pm 5.357$ ) on the basis of observation of these parameter shows significant value in  $CCl_4 + C. tinctoria$  50mg/kg,  $CCl_4 + C. tinctoria$  100mg/kg,  $CCl_4 + Silymarin$  25mg/kg group in compare to normal control group, the fourth group  $CCl_4 + C. tinctoria$  100mg/kg,  $CCl_4$  was shows more significant value in compare to standard and control groups so this dose of hydro-alcoholic extract of *C.tinctoria* was more effective in compare to low dose. Morphological parameter was observed and concluded that the animal body wt. of these group  $CCl_4 + C. tinctoria$  50mg/kg ( $191.54\pm 7.98$ ),  $CCl_4 + C. tinctoria$  100mg/kg ( $176.90\pm 7.87$ ),  $CCl_4 + Silymarin$  25mg/kg ( $185.86\pm 6.71$ ) group in was shows significance value in compare to normal control group that means the final body wt. of animal was decreases in compare to initial body wt. of animal and group fourth shows the more significant value in compare to normal and standard control group so group fourth  $CCl_4 + C. tinctoria$  100mg/kg ( $176.90\pm 7.87$ ) was more effective in compare to low dose. The liver wt. of experimental animals was increases because of hepatotoxicity induced by  $CCl_4$  but after treating with sylimarin and hydro-alcoholic extract of *C.tinctoria* low dose (50mg/kg/b.w) ( $178.72\pm 8.00$ ) and high dose (100mg/kg/b.w) ( $194.93\pm 7.48$ ) the wt. of liver was increases in compare to initial liver wt. and group fourth was shows more significant value in compare to low dose of *C.tinctoria*. Histopathological study on the basis of result observation it shows that the normal control group section was shows the clear well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein, the second group was shows fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis and presence of fatty cyst, the third group which was treated with *C. tinctoria* 50mg/kg this group was observed the exhibited significant liver protection against the toxic substance as evident by normal lobular pattern with a mild degree of fatty change, absence of necrosis and lymphocyte infiltration mild comparable to the standard and control, the fourth treated group was  $CCl_4 + C. tinctoria$  100mg/kg this group was observed the liver architecture more effective than low dose and almost comparable to the standard and control. However, accumulation of fatty lobules (steatosis), necrosis and

scattered lymph mononuclear (LMN) cell infiltrate in hepatic parenchyma were noticed. Fifth group was treated with standard sylimarin which shows the approximately similar to that of control group, so the group fourth high dose of hydro-alcoholic extract of *C.tinctoria* was shows more effective histopathology in compare to low dose. To conclude this study the high dose of hydro-alcoholic extract of *C.tinctoria* (100mg/kg/b.w.) was more effective and low dose also give hepatoprotective effect but it shows less effective in compare to standard and control group. Hepatotoxicity is the foremost health troubles over all worlds with the confluences of liver cirrhosis and drug induced liver injury which is leading cause of death in western and developing countries. There is urgent need of the health professionals and scholars working in the field of pharmacology to develop an alternative medicine or diagnostic aids to cure different kinds of liver diseases spreaded in worldwide.

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