



## Screening of Leaves and Roots of *Eclipta alba* for Hepatoprotective Activity

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### Abstract

Leaves and roots are used as hepatic tonic rejuvenates hair, cirrhosis, ear aches, hepatitis, headache and enlarged spleen. Studies on the hepatoprotective activity on methanolic extract and sub fractions of leaves and the chloroform extract and sub fractions of roots of *Eclipta alba* was carried out using carbon tetrachloride- induced liver damage and Lysosomal enzymes level in wistar albino rats. The methanolic extract of leaves and the chloroform extract of roots of *Eclipta alba* showed significant activities ( $P<0.01$ ) and ( $P<0.05$ ) respectively causing 72.8% & 47.96% reduction of lysosomal enzyme. The triterpenoid Eclabasaponin fraction from methanolic extract of leaves produced significant ( $P<0.001$ ) (78.78%) and the alkaloidal fraction ( $P<0.05$ ) (60.65%) reduction of carbon tetra chloride induced increase in lysosomal enzyme in blood. The Coumestan fraction (CF) and Triterpenoidal saponin fraction (RTF) from the chloroform extract of roots produced very significant ( $P<0.01$ ) (75.6%) and ( $P<0.05$ ) (52.41%) respectively reduction of carbon tetra chloride induced increase in lysosomal enzyme levels in blood.

**Key Words-** Carbon tetrachloride, Lysosomal enzymes, Silymarin. *Eclipta alba*.

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### Introduction

*Eclipta alba* Linn; Family: Compositae (Asteraceae) is known by other names: Bhangra, Bhringraj, Mochkand, and Maka and is found throughout India & Southwestern U.S. [10]. The plant contains the alkaloid ecliptine; other chemicals identified are wedelolactone, demethylwedelolactone, wedelic acid, apigenin, luteolin, b-amyrin, etc [3]. Wedelolactone and demethylwedelolactone have potent trypsin inhibitory effect [4]. Diuretic, hypotensive and hypocholesterolemic effect of *Eclipta alba* in mild hypertensive subject were reported by Rangineni V. et al and some other pharmacological activities reported Neuropharmacological profile of *Eclipta alba* (Linn.) Hassk [14], Antihyperglycemic activity of *Eclipta alba* leaf on alloxan-induced diabetic rats [2], Analgesic studies on total alkaloids and alcohol extracts of *Eclipta alba* [12], Eclalbatin, a triterpene saponin from *Eclipta alba*, DNA-damaging steroidal alkaloids from *Eclipta alba* from the suriname rainforest [1], Chemical constituents

of *Eclipta alba* (L.) Hassk [15], Phenolics and other constituents from *Eclipta alba* [8], Ecliptal, a new terthienyl from *Eclipta alba* [13].

## Materials and Methods

### Plant material

Aerial parts of the plant *E.alba* were collected from medicinal garden of BBDNITM and authenticated by pharmacognostic and voucher sample duly authenticated from Taxonomic division NBRI, Lko. & a sample kept for future reference (Voucher No: NBRI/CIF/Re./08/2008/32).

### Animals

Healthy male or female albino Wister rats each weighing 150-200 g were used for study. The rats were housed in polypropylene cages and maintained under standard conditions (12 hr light and dark cycles, at  $25\pm 3^{\circ}\text{C}$  and 35-60% humidity). Standard palletized feed and tap water were provided ad libitum. All animal experiments were carried out as per CPCSEA guidelines (Approval No. BBDNITM/IAEC/clear/11/2008).

### Preparation of extracts and Fractionation and isolation of phytoconstituents fraction

A definite weight of crude drug powder (powder of leaves and roots) was taken in a soxhlet apparatus thimble after making moderately coarse and then continuous hot soxhlet extraction was done by various solvent in a sequence of increasing polarity as follows:

1. Petroleum ether
2. Acetone
3. Chloroform
4. Methanol
5. Water

With the help of Phytochemical tests, methanolic extract of leaves and chloroform extract of roots were show the positive tests for alkaloid, triterpenoid and flavanoids (Coumestone). Mainly various chemical constituents of *Eclipta alba* comes in alkaloids, triterpenoids and flavanoids categories as responsible for hepatoprotective activity.

### Fractionation of methanol extract of leaves of *E. alba*

Methanolic extract showed significant activity and so was taken up for column chromatography and fractionation. A fixed quantity of extract was chromatographed on silica gel (60/120) column with methanol as the stationary phase. After addition of the extract, proper time was allowed for partitioning of the constituents over the column (15min). First elution was done with chloroform (50ml) The chloroform was added to ht column and after allowing ten min. for stabilization, ten ml fractions were collected in labeled test tubes at the rate of 20 drops /min. Each fraction was subjected to chemical test and T.L.C. was performed to identify nature and number of constituents in the fractions and ensure proper separation of constituents. For T.L.C., mobile phase used was chloroform: benzene (9:1). Subsequent elutions were done with chloroform: methanol (70:30), chloroform: methanol (40:60) and finally Methanol taking 50 ml of each and collecting five fractions of ten ml each and subjecting fractions to chemical tests and T.L.C. All fractions showing single component or single spot T.L.C. were also scanned for U.V. $\lambda_{\text{max}}$  (Shimadzu) to investigate nature of constituents. All subsequent fractions showing single component with similar Rf values were combined and worked upon for precipitation of the components by using suitable solvent or

pH combinations. All precipitated compounds were subjected to I.R. (Shimadzu) to confirm nature of functional groups.

#### ***Fractionation of chloroform extract of roots of E. alba***

Fractionation of chloroform extract was similarly done as above on silica gel (60/120) column with chloroform as stationary phase. First elution was done using petroleum ether and collecting 10ml fractions and later by petroleum ether: chloroform (70:30), (40:60) and then by chloroform. Chemical tests and T.L.C. were performed for fractions to identify nature and number of components as earlier and then U.V. scanning for all single component fractions was performed. Precipitation was done as earlier by combining subsequent fractions showing similar Rf values [9].

#### **Toxicity studies**

##### ***Acute toxicity study***

Albino Rats of either sex weighing between 200 -250 grams were used in the present investigation. The animals were fasted for an overnight prior to the experimental procedure. The 'Up and Down' or 'Staircase' method was adopted, and dose of Methanolic extract was administered. Two Rats were orally dosed, say 250mg/kg and observed for a period of 24hours for any mortality. The subsequent doses were then increased by a factor 1.5, if the dose is tolerated, or decreased by factors 0.7 if it is lethal. The maximum non-lethal and the minimum lethal dose are thus determined using only about 10 mice. Once the approximate LD<sub>50</sub> or the range between the maximum non – lethal and minimum lethal dose was found, a final and more reliable LD<sub>50</sub> assay was planned using at least 3 or 4 dose levels within this range with more number of animals in each group. LD<sub>50</sub> is expressed in term of mg/ kg. In addition, source of animal, sex, age, body weight, oral time and solvent, and presence and absence of any immediate reaction were also recorded for further references. The maximum non-lethal dose was found to be 5000mg / kg body weight, hence 1/10<sup>th</sup> of which was taken as effective dose (500mg / kg body weight) for various extracts [7].

##### ***Carbon tetrachloride-induced hepatotoxicity***

Rats were fasted for 16hr, and then divided into several groups of eight each. Groups 1 was control group receiving normal saline only (10 mg/per kg i.p) carbon tetrachloride will be administered to animals of the other groups by subcutaneous injection at the back. The test drug at varying concentration depending on the design of the experiment was administered and the reference drug was administered to different groups at two hour before and 24 and 48 hour after carbon tetrachloride administration. In the last two group's only carbon tetrachloride and the vehicle was given with no additional treatment. All animals were killed 72 hours after carbontetrachloride administration, whole blood was drawn from the carotid artery and the serum was separated for different assays. Liver tissues were removed, and then liver sections was taken from each lobe of the liver and fixed in 10% neutral formalin. Then 72 hour after carbon tetrachloride injection there was usually significant increase in GOT, GPT, serum albumin, ALP relative to control group. Rats that received hepaoprotective drugs should significantly decrease GOT, GPT, serum albumin, ALP. This phenomenon was also conformed by histological observation [11].

##### ***Carbon tetra chloride induced lysosomal enzyme inhibitory activity***

The animals weighing 150-200gm were taken for study. They were divided in seven groups having six rats in each. Group I served as control, given mixture of 2.5% DMSO and 2.5% Tween-80 in water. Group II served as Diseased and given carbon tetra chloride (0.5ml/kg body weight i.p.). Group III served as reference and received silymarin 50 mg/kg body

weight. Groups IV, V, VI, and VII were given extracts and fractions (250 mg/kg body weight) in mixture of 2.5% DMSO and 2.5% Tween-80 in water orally. For this study the animals were kept on fasting for 16 hrs prior to induce disease. The carbon tetra chloride was given for 5 consecutive days, after that, the extracts were given for 7 consecutive days. After 24 hrs last dose given, the blood was withdrawn and the serum was subjected to serum enzymes estimation [6].

### **Biochemical Estimations**

The activity of lysosomal enzymes was investigated in blood serum. The blood withdrawn was centrifuged for 10 minutes at 2000 r.p.m. and separated plasma was taken for estimation of cathepsin which corresponds to lysosomal enzyme activity.

### **Determination of cathepsin enzymes:**

#### **Reagents required:**

- 1- Buffered hemoglobin substrate (pH 3.6) [15% hemoglobin solution]
- 2- 10% tri chloro acetic acid
- 3- Follin's phenylciocaltau reagent:
- 4- 5% sodium hydroxide solution
- 5- Standard tyrosine solution in 0.1N HCl (10 mg/100ml)
- 6- 1% alkaline copper sulphate

#### **Procedure**

0.9 ml of hemoglobin solution was added to 0.5 ml of plasma from various groups of animal and incubated at 37°C for 2 hrs after then 1 ml of 10% Tri chloro acetic acid was added and centrifuged at 2000 r.p.m for 10 minutes. Supernatant liquid was separated and taken 1.5 ml of it then 1 ml of 5% NaOH, 4.5 ml of 1% alkaline copper sulphate and 0.5 ml of Follin's reagent was added. A blank solution containing hemoglobin solution, alkaline copper sulphate, tri-chloro acetic acid and Follin's reagent was also prepared in another test tube and incubated same way. After 20 minutes the absorbance was measured at 620 nm for all. In the reaction the lysosomal enzymes particularly cathepsin present in the blood of various animal groups acts on hemoglobin and results in to free tyrosine whose absorbance is measured at 620 nm after developing with Follin's reagent. For animal groups in which tyrosine absorbance readings are higher indicate higher level of cathepsin indicating higher level of lysosomal enzyme activity. Animals treated with reference or therapeutically active fractions should show less tyrosine readings and lysosomal enzyme activity.

Lysosomal inhibiting activity was expressed as percentage inhibition and estimated by following formula reported in the Reference.

$$\% \text{ Inhibition of lysosomal enzymes} = \frac{A_{(\text{disease control})} - A_{620}}{A_{(\text{disease control})}} \times 100$$

#### **Statistical analysis**

One way analysis of variance (ANOVA) followed by dunnett's test, was carried out & p< 0.05 was considered as significant. Groups were compared with control group.

## Results and Discussion

Methanolic extract fractions of leaves and chloroform extract fractions of roots (*Eclipta alba*) showed significant hepatoprotective activity as investigated by using Carbon tetra chloride models. The other solvent extracts of the leaves did not show significant hepatoprotective activity. It was observed that Eclalbasaponin triterpenoidal fraction from leaves most significant in hepatoprotective activities against carbon tetra chloride and acetaminophen induced hepatic injury as so on by normalization of various hepatic enzymes levels and lysosomal enzyme levels. The Coumestan fraction (CF) from roots also showed significant activities but less than the triterpenoidal Eclalbasaponin fraction (LTF) from methanolic extract of leaves in various models.

**Table: 1 Effect of various solvent extracts and fractions from *Eclipta alba* leaves & roots on biochemical parameters in Carbon tetra chloride induced hepatic injury in Rats**

S. No.	Group	Dose	S.G.P.T	S.G.O.T	A.L.P	Bilirubin
1.	Group 1	0.5ml	190.4±4.7	60.5±14.0	81.6±12.4	0.78±0.03
2.	Group 2	2ml/kg	412.7±15.8	210.0±26.0	195.4±18.5	0.97±0.04
3.	Group 3	100ml/kg	210.7±4.4 ***	97.0±25.0 **	86.6±17.0 ***	0.40±0.06 **
4.	Group 4	200	374.0±21.0	128.0±23.0 **	126.0±21.0 **	0.79±0.06 *
5.	Group 5	200	290.0±5.5 **	106.7±17.5 **	119.0±23.0 **	0.77±0.05 *
6.	Group 6	150	254.0±24.4 **	62.2±18.0 ***	97.0±3.3 ***	0.75±0.02 *
7.	Group 7	150	300.0±21.2 *	135.0±13.8 *	132.0±12.1	0.82±0.02
8.	Group 8	150	192.0±29.4 ***	62.0±19.0 ***	94.2±4.3 ***	0.72±0.03 *
9.	Group 9	150	310.0±20.1 *	140.0±14.1 *	154.0±12.0 *	0.81±0.03

**Group 1**= Normal Control, **Group 2**= Disease Control, **Group 3**= Disease animals treated with reference drug silymarin, **Group 4**= Disease animals treated with chloroform extract of roots **Group 5**= Disease animals treated with methanolic extract of leaves, **Group 6**= Disease animals treated with Coumestone Fraction (CF), **Group 7**= Disease animals treated with triterpenoidal fraction (RTF), **Group 8**= Disease animals treated with triterpenoidal fraction (LTF), **Group 9**= Disease animals treated with Alkaloidal fraction (AF) of leaves

N= 6 samples in each group; Values are expressed as Mean±SEM

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001 when compared with disease control

### **Effect of extracts & fractions on carbon tetra chloride induced hepatic damage**

Carbon tetra chloride (CCl<sub>4</sub>) induced hepatic damage causes leakage of SGOT, SGPT, Serum ALP, and serum bilirubin etc. It also causes changes in the cell membrane ratios of cholesterol-phospholipids and sphingomyelins- phosphatidyl cholin (16). These changes lead to large scale morphological damages in liver hepatocytes. The methanolic extract of leaves of *Eclipta alba* and chloroform extract of roots, both showed significant activity (p<0.01-0.001) and (p<0.01) respectively, against CCl<sub>4</sub> induced increases in hepatic enzymes. The methanolic extract from leaves showed significant (p<0.01) reduction in carbon tetra chloride induced increase in SGPT level & reduction (p<0.05) in serum in serum bilirubin, whereas the chloroform extract of roots showed reduction (p<0.05) of serum bilirubin and non-significant reduction of SGPT.

**Carbon Tetra Chloride Induced Histological Changes**

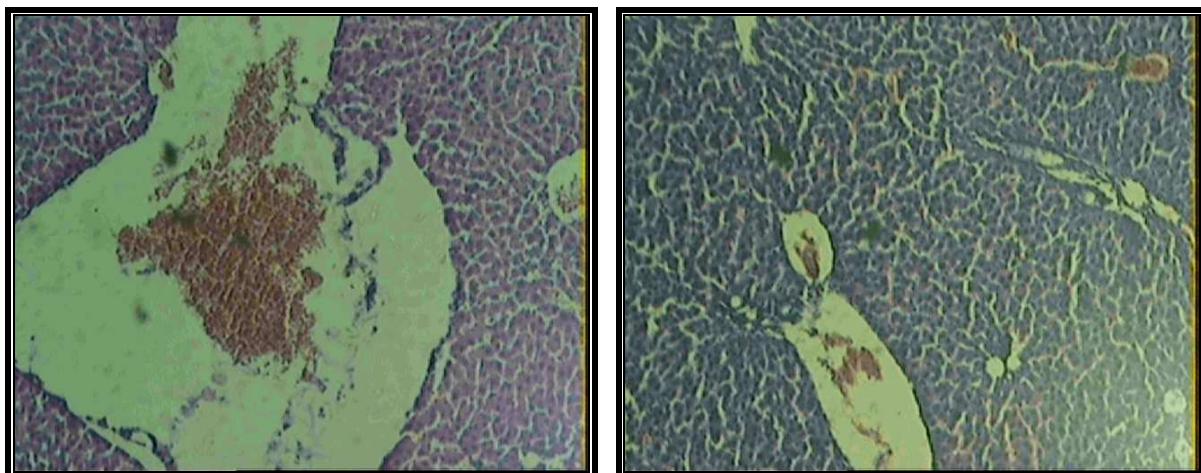


Fig: 1 Histological Section-Liver: Disease control group

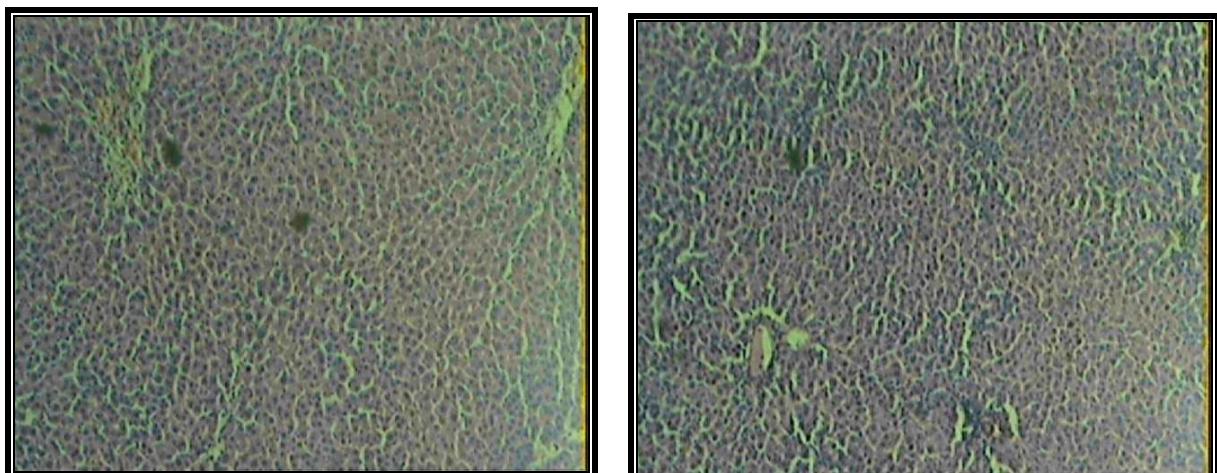


Fig: 2 Histological Section-Liver: Normal group

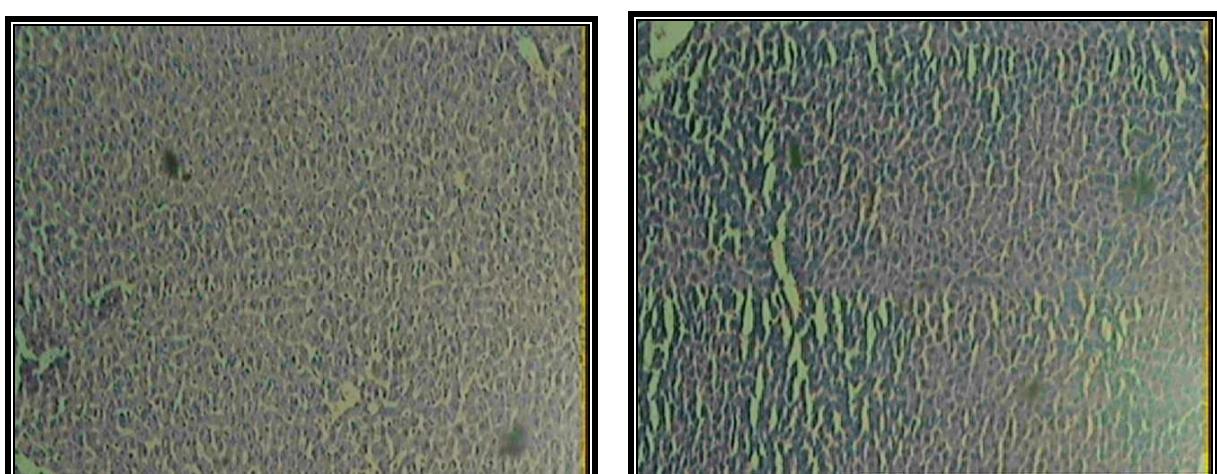


Fig: 3 Histological Section-Liver: silymarin treated group

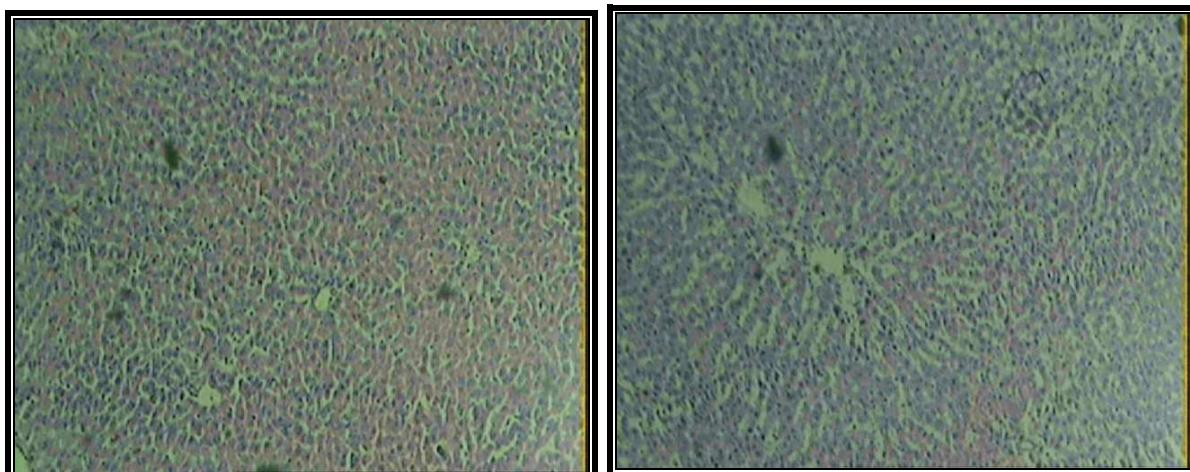


Fig: 4 Histological Section-Liver: Methanolic extract of leaves fraction (LTF)



Fig: 5 Histological Section-Liver: Chloroform extract of root fraction (RTF)

The Coumestan fraction (CF) from chloroform extract of roots of *E.alba* showed significant reduction ( $p<0.01$ ) of SGPT, ( $p<0.001$ ) of SGOT & ALP and ( $p<0.05$ ) of serum bilirubin level. The Triterpenoidal Eclabasaponin fraction from chloroform extract of roots of *E.alba* was less significant and caused slightly significant ( $p<0.05$ ) reduction of serum SGPT & SGOT and insignificant reduction of ALP and serum bilirubin levels. The Triterpenoidal Eclalbasaponin fraction (LTF) from methanolic extract of leaves of *E.alba* showed most significant activity in reduction of various hepatic enzyme concentration in the blood ( $p<0.001$ ) and was most effective as compared to other fractions. The alkaloidal fraction (AF) from the methanolic extract of the leaves was only slightly significant ( $p<0.05$ ) in reduction of SGPT, SGOT & ALP in animal induced with carbon tetra chloride hepatic damage and no reduction of serum bilirubin levels. The activity of the extract and fractions may thus be because of stabilization of hepatocyte membrane and thus reduction in leakage of SGOT, SGPT, albumin etc. in to the plasma. CCl<sub>4</sub> induced hepatocyte damage also causes damage to the lysosomal membrane causing liberation of degradative enzymes like acid phosphatase, cathepsin etc followed by cell destruction [5].

### Lysosomal enzymes inhibition activity

**Table 2: Lysosomal enzymes inhibition produced by various extracts & fractions from *E. alba* leaves & roots**

S. No.	Test Group	Dose	Absorbance ( $\lambda_{\text{max}} 620\text{nm}$ )	% inhibition
1	Group 1	0.5ml	0.108	-
2	Group 2	2ml/kg	0.788	-
3	Group 3	100ml/kg	0.135	82.9***
4	Group 4	200	0.215	72.8**
5	Group 5	200	0.410	47.96*
6	Group 6	150	0.170	78.42***
7	Group 7	150	0.310	60.65*
8	Group 8	150	0.192	75.6**
9	Group 9	150	0.372	52.41*

**Group 1**= Normal Control, **Group 2**= Disease Control, **Group 3**= Disease animals treated with reference drug silymarin, **Group 4**= Disease animals treated with methanolic extract of leaves, **Group 5**= Disease animals treated with chloroform extract of roots **Group 6**= Disease animals treated with Alkaloidal fraction (AF) of leaves, **Group 7**= Disease animals treated with triterpenoidal fraction (LTF), **Group 8**= Disease animals treated with Coumestone Fraction (CF), **Group 9**= Disease animals treated with triterpenoidal fraction (RTF),

N= 6 samples in each group

Values are expressed as Mean $\pm$ SEM

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001 when compared with disease control

### *Effect of extracts & fractions on carbon tetra chloride induced changes in Lysosomal enzyme levels*

The methanolic extract of leaves of *Eclipta alba* and the chloroform extract of roots of *Eclipta alba* showed significant activities (P<0.01) and (P<0.05) respectively causing 72.8% & 47.96% reduction of lysosomal enzyme. The Triterpenoidal Eclalbasaponin fraction from methanolic extract of leaves produced significant (P<0.001) (78.78%) and the alkaloidal fraction (P<0.05) (60.65%) reduction of carbon tetra chloride induced increase in lysosomal enzyme in blood. The Coumestan fraction (CF) and Triterpenoidal saponin fraction (RTF) from the chloroform extract of roots produced very significant (P<0.01) (75.6%) and (P<0.05) (52.41%) respectively reduction of carbon tetra chloride induced increase in lysosomal enzyme levels in blood.

### Conclusion

Further studies need to be carryout to isolate compounds in appreciable quantities and subjected to detailed dose response relationship studies and preclinical toxicity studies as important lead compound for further QSAR study and clinical trials.

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