Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 877 - 884

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ECLIPTA ALBA AMELIORATES CARBON TETRACHLORIDE-INDUCED LIPID PEROXIDATION: AN *IN VITRO* STUDY

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ABSTRACT

The present investigation was an attempt to evaluate the possible ameliorative effect of *Eclipta alba* on carbon tetrachloride (CCl_4) – induced lipid peroxidation in liver of mice *in vitro*. Antioxidant activity of medicinally important plant *Eclipta alba* was determined by DPPH – radical scavenging activity. Liver tissue was collected from healthy Swiss albino female mice weighing 30-35 gm. Liver homogenates were prepared and used for various treatments (CCl_4 with and without hydro-alcoholic extract of *Eclipta alba*) followed by analysis of lipid peroxidation and protein content. The results revealed that addition of CCl_4 (5-50 µg/ml) to liver homogenate caused a significant increase in lipid peroxidation which was maximum at 10 µg/ml. An addition of hydro-alcoholic extract of *Eclipta alba* (25-150 µg/ml) significantly reduced CCl_4 (10 µg/ml) - induced lipid peroxidation in concentration-dependent manner. The result was compared with standard drug Liv. 52. The study confirmed the ameliorative effect of the hydro-alcoholic extract of *Eclipta alba* even higher than the Liv. 52, which may be attributed due to its antioxidative property as revealed by DPPH radical scavenging activity measured.

KEYWORDS: **Carbon tetrachloride, *Eclipta alba*, Lipid peroxidation, Liver, Mice**

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INTRODUCTION

The liver performs many functions vital to the health of the organism. The liver transforms and excretes many drugs and toxins. These substances are frequently converted to inactive forms by reactions that occur in hepatocytes [1, 2]. Inspite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver functions and offer protection to the hepatocytes from damage. Therefore, many folk remedies from plant origin are evaluated for their possible antioxidant and hepatoprotective effect against chemically induced liver damage in experimental animals. Plant derived natural products such as flavonoids, terpenoids, steroids, etc. have diverse pharmacological properties including antioxidant properties. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyland hydroxyl radicals etc., thereby preventing or delaying damage to the cells and tissues. Antioxidant compounds have potential to mitigate the effect of free radicals and play an important role as a health protecting factor [3]. Carbon tetrachloride induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts [4]. CCl_4-induced hepatotoxicity resulted in oxidative stress and lipid peroxidation. CCl_4 was the first toxin for which it was shown that the injury produced is largely or entirely mediated by a free-radical mechanism. It is believed that CCl_4 is metabolized by the cytochrome P_{450} system to give the trichloromethyl radical, a carbon-centred radical. Covalent binding of trichloromethyl free radicals to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation (LPO) and finally to cell apoptosis and necrosis[5,6,7] High levels of reactive oxygen species (ROS) damage cells and are involved in several human pathologies, including liver cirrhosis and fibrosis. The second phase of CCl_4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators [8]. Hence, CCl_4 – induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver [1]. *Eclipta alba* (Linn.) Hask. of the Asteraceae family, is a well-known herb in the Indian system of medicine. Alcoholic extracts of the plant shows protective effect on experimental liver damage in rats and mice [9]. It is good for blackening and strengthening of the hair, for stopping haemorrhages and fluxes and for strengthening of gums. Root is used as emetic and purgative. It is applied externally as antiseptic to ulcers and wounds. Also used as tonic, spasmogenic, ovicidal and in healing of fractures [10]. The purpose of the present study was to evaluate the *in vitro* ameliorative effect of *Ecliptaalba*against CCl_4-induced lipid peroxidation in liver of mice.

MATERIALS AND METHODS

Plant material

Eclipta alba (Bhringraj), the whole plant material was collected from the Botanical garden of Gujarat University, Ahmedabad, India and was authenticated by the Botany Department, School of Sciences, Gujarat University, Ahmedabad, India.

Extract preparation

The extract was prepared according to World Health Organization protocol CG-06 with slight modifications [11]. 5 gm. of shade dried powdered material of plant was extracted overnight by soaking method using 100 ml of 50% aqueous-alcoholic solvent. The content was filtered successively through ordinary and then Whatman filter paper No. 1. Extraction procedure was repeated. Both the fractions were pooled, dried and stored in a dark bottle at 4 ^{o} C. During the experiment known amount of dried extract was redissolved in double distilled water and used.

Chemicals

Analytical grade chemicals used in entire study were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India, Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Sigma-Aldrich, St. Louis, MO, USA.

Olive oil was obtained from Figaro, Madrid, Spain.

Animals

Healthy, adult, pathogen free, colony bred Swiss female albino mice (*Mus musculus*) weighing between 30-35 gm. (6-8 weeks old) used in the present study were obtained from Zydus Research Centre, Ahmedabad, India. The animals were housed under controlled conditions (temperature 25±2 ◦ C; relative humidity 50-55%; 12 h light/dark cycle) in the animal house of the Zoology Department, Gujarat University, Ahmedabad, India. Animals of different experimental groups were caged separately and maximum of five animals per cage were maintained on certified pelleted rodent feed supplied by Amrut feeds, Pranav Agro Industries Limited, Pune, India and potable water adlibitum. The experimental procedures were assessed and approved by "The Committee for the Purpose of Control and Supervision of Experiments on Animals" (Reg-167/1999/CPCSEA), New Delhi, India. Guidelines for Care and Use of Animals in Scientific Research 1991 published by Indian National Sciences Academy, New Delhi, India were followed.

DPPH radical scavenging activity [12]

The free radical scavenging activity of plant extract of *Eclipta alba* was done according to the method as reported by Gyamfi *et al.*[12]. 50 µl of the plant extract in methanol, yielding 100 µg/ml respectively in each reaction was mixed with 1 ml of 0.1 mM 1, 1-Diphenyl–2-picrylhydrazyl(DPPH) in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) only was used as control of experiment. After 30 min. of incubation at room temperature the reduction of the DPPH free radical was measured reading the absorbance at 517 nm. BHT was used as control.

The percent inhibition was calculated from the following equation:

% Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of control] × 100

Experimental Design

Animals were sacrificed and liver was dissected out, blotted free of blood, quickly weighed and used for the experiments.

Lipid peroxidation activity [13]

The level of lipid peroxidation in the liver was measured by the method as described by Ohkawa *et al*. with slight modification [13]. This method is based on the formation of a red chromaphore that absorbs light at 532 nm following the reaction of thiobarbituric acid (TBA) with products of lipid peroxidation like malondialdehyde (MDA) and others collectively called as thiobarbaturic acid reactive substances (TBARS). The results were expressed as n moles MDA formed/mg protein/60 min.

The experiment was set as mentioned below.

Liver homogenates (10%) were prepared in 0.1 M phosphate buffered saline (pH 7.4).

Following sets of tubes were prepared.

(A) Control tubes containing 0.2 ml of homogenate only.

(B) Di methyl sulfoxide (DMSO) Control tubes containing 0.2 ml DMSO only.

(C) Reaction mixture contained 0.2 mL of homogenate and varying concentrations of CCl_4 (0.4% V/V) solution (5-50 µg/ml)) in DMSO.

(D) Antidote control tubes containing *Eclipta alba* extract (150 µg/ml) only.

(E) Set of tubes containing *Eclipta alba* extract of different concentrations (25-150 µg/ml) along with CCl_4 (10 µg/ml).

The final volume (1.0 ml) was adjusted with 0.1 M phosphate buffered saline. Reaction was initiated by addition of H_2O_2 and the mixture was incubated at 37 ◦ C for 30 min. with occasional shaking [14]. Lipid peroxides (TBARS) were estimated by using 8.1% sodium dodecyl sulphate, 20% acetic acid and 1% thiobarbituric acid solution. Care was taken to adjust the pH of 20% acetic acid to 3.5 using 1 M NaOH. The blank for each sample was prepared by substituting the TBA solution with distilled water. The solution was mixed and heated in a water bath at 95 ◦C for 60 min. The tubes were immediately cooled and aliquots were centrifuged at 1000 g for 10 min. The absorbance of the resulting supernatant fraction was read at 532 nm

against blank on a Systronics 118 UV-Vis spectrophotometer.

Protein content [15]

Protein content was estimated in liver by the method of Lowry *et al*. using bovine serum albumin as a standard [15]. When protein reacts with phenol reagent of Folin Ciocalteu, a deep blue colour develops. The colour development is due to two reactions occurring simultaneously i.e., the reaction of alkaline copper sulphate solution with peptide bonds and reduction of phosphomolybdic and phosphotungstic acids present in the protein. The blue colour that develops is quantitatively proportional to the total protein, which was measured at 540 nm. The protein content was expressed as mg/100 mg tissue weight.

The experiment was set as mentioned below.

Liver homogenate was prepared by using 100 mg liver tissue in 5 ml of double distilled water. Reaction mixture contained 0.2 ml of homogenate and varying concentrations of CCl_4 (0.4% V/V) solution (5-50 µg/ml)) in DMSO.

Following sets of tubes were prepared:

(A) Control tubes containing 0.2 ml of homogenate only.
(B) DMSO control tubes containing 0.2 ml DMSO only.
(C) Reaction mixture contained 0.2 mL of homogenate and varying concentrations of CCl_4 (0.4% V/V) solution (5-50 µg/ml)) in di methyl sulfoxide (DMSO).
(D) Antidote control tubes containing *Eclipta alba* extract (150 µg/ml) only.
(E) Set of tubes containing *Eclipta alba* of different concentrations (25-150 µg/ml) along with CCl_4 (10 µg/ml).
(F) The standard tube contains 0.2 ml of bovine serum albumin in (mg/ml) concentration substituted by liver homogenate.

The final volume (1.0 ml) was adjusted with distilled water [14].

Reaction mixture was incubated at 37 ◦ C for 30 min. with occasional shaking. After incubation, 4.0 ml of alkaline copper sulphate solution was added and incubated at 37 ◦ C for 15 min. and then 0.4 ml of Folin Ciocalteu reagent was added and incubated for 30 min. at room temperature. The absorbance of the resulting supernatant fraction was read at 540 nm against blank on a Systronics 118 UV-Vis spectrophotometer.

STATISTICAL ANALYSIS

The results are expressed as means ± S.E.M. Hypothesis testing method included one way Analysis of Variance (ANOVA) followed by Tukey test. The level of significance was accepted with $^{*}p<0.05$.

RESULTS

Addition of CCl_4 (5-50 µg/ml) to liver homogenate significantly ($p<0.05$) increased H_2O_2-induced lipid peroxidation *in vitro* . At 10 µg/ml CCl_4 concentration, maximum lipid peroxidation was observed in liver homogenate. Further, increase in CCl_4 concentrations caused lesser lipid peroxidation, as compared to 10 µg/ml, though it was always significantly higher than the control (Table 1). Addition of CCl_4 (5-50 µg/ml) to liver homogenate significant decreases protein content *in vitro* as compared to control. Effect was dose-dependent (R^2 value = 0.9854) up to 50 µg/ml concentration (Table 1). Concurrent addition of hydro-alcoholic extract of *Eclipta alba* (25-150 µg/ml) along with CCl_4 (10µg/ml) significantly ($p<0.05$) retarded CCl_4- induced lipid peroxidation in liver homogenate. The effect was concentration-dependent (*Eclipta alba,* R^2 value = 0.9201) with maximum retardation at 150 µg/ml of hydro alcoholic extract of *Eclipta alba*. An addition of only hydro-alcoholic extract of *Eclipta alba* (control) did not cause any significant increase in lipid peroxidation (Table 2). The result was also compared with standard drug Liv. 52. *Eclipta alba* was found to be more effective than Liv.52. The result shown in Table 2 revealed amelioration in concentration of total protein content in liver homogenate treated with CCl_4 along with hydro-alcoholic extract of *Eclipta alba*. The DPPH radical-scavenging activity of *Eclipta alba* is shown in Figure 1. It was observed that the DPPH free radical scavenging activity was concentration-dependent, with a maximum inhibition (%) of 82.47% at a concentration of 250 µg/ml for hydro-alcoholic extract of *Eclipta alba*. No

difference in inhibition was noted with further increase in concentration of the *Eclipta alba* extract. The DPPH free radical-scavenging activity of Butyl Hydoxy Toluene (BHT) is also presented in Figure 1. The scavenging activity was concentration-dependent and maximum scavenging activity was 97.10%. The IC_{50}values was calculated. The IC_{50}value for *Eclipta alba* was 50 µg/ml.

Table 1
Effect of carbon tetrachloride (CCl_4) on lipid peroxidation and protein content in liver homogenate

CCl_4 (µg/ml)	Lipid peroxidation (nmole MDA formed/mg protein/60 min.)	Protein content (mg/100 mg tissue weight)
0	2.96 ± 0.11	12.74 ± 0.16
5	22.98 ± 0.68*	8.86 ± 0.22*
10	34.60 ± 0.63*	7.98 ± 0.18*
20	31.51 ± 1.01*	6.77 ± 0.12*
30	25.71 ± 0.82*	5.87 ± 0.15*
40	23.24 ± 0.58*	5.06 ± 0.14*
50	21.83 ± 0.68*	4.20 ± 0.17*

Results are expressed as Mean ± S.E.M., n = 10.
**** p<0.05 as compared to Control***
Level of significance p < 0.05.

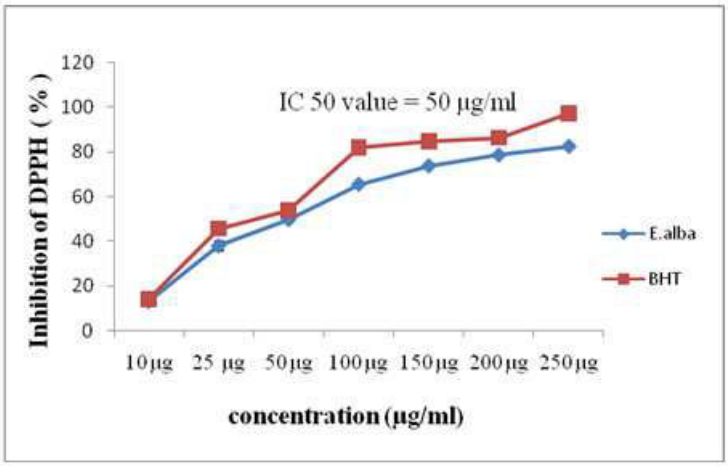

Figure 1
DPPH radical scavenging activity of the hydro-alcoholic extract of Eclipta alba.

Table 2

Effect of hydro-alcoholic extract of Eclipta alba on carbon tetrachloride (CCl_4) -induced lipid peroxidation and protein content in liver homogenate.

CCl_4 + Plant extract (µg/ml)	Lipid peroxidation (nmole MDA formed /mg Protein /60 min.)	Protein content (mg / 100 mg Tissue weight)
CONTROL		
Untreated Control	2.96 ± 0.11	12.74 ± 0.47
Antidote Control *Eclipta alba*(150 µg/ml)	3.03 ± 0.27	12.93 ± 0.79
Antidote control Std. Liv. 52(150 µg/ml)	3.08 ± 0.08	12.35 ± 0.13
Carbon Tetrachloride(CCl4) treated		
CCl_4 (10µg/ml)	34.61 ± 0.63	7.99 ± 0.18
CCl4 + *Eclipta alba* (E.A.) plant extract treated		
CCl_4 (10 µg/ml) + 25 µg/ml E.A.	26.83 ± 0.60^{*a}	8.55 ± 0.11*
CCl_4 (10 µg/ml) + 50 µg/ml E.A.	20.58 ± 0.97^{*a}	8.95 ± 0.34*
CCl_4 (10 µg/ml) + 75 µg/ml E.A.	11.25 ± 0.66^{*a}	9.70 ± 0.54*
CCl_4 (10 µg/ml) + 100 µg/ml E.A.	8.24 ± 0.34^{*a}	10.01 ± 0.34^{*a}
CCl_4 (10 µg/ml) +125 µg/ml E.A.	5.57 ± 0.18^{*a}	10.70 ± 0.30^{*a}
CCl_4(10 µg/ml) + 150 µg/ml E.A.	3.77 ± .023^{*a}	11.45 ± 0.50^{*a}
CCl4 + Standard drug Liv. 52 treated		
CCl_4 (10 µg/ml) + 25 µg/ml Liv.52	32.31 ± 0.33	7.80 ± 0.10*
CCl_4 (10 µg/ml) + 50 µg/ml Liv.52	25.18 ± 0.73*^{a}	8.28 ± 0.24*
CCl_4 (10 µg/ml) + 75 µg/ml Liv.52	19.11 ± 0.84^{*a}	8.81 ± 0.10*
CCl_4 (10 µg/ml) +100 µg/ml Liv.52	12.79 ± 0.50^{*a}	9.32 ± 0.23^{*a}
CCl_4 (10 µg/ml) +125 µg/ml Liv.52	9.10 ± 0.16^{*a}	10.24 ± 0.13^{*a}
CCl_4 (10 µg/ml) +150 µg/ml Liv.52	4.86 ± 0.12^{*a}	11.06 ± 0.12^{*a}

Results are expressed as Mean ± S.E.M., n = 10.
No significant difference was noted between control tubes.
* ***p<0.05 as compared to Control***
^{a} ***p<0.05 as compared to Toxin treated***
Level of significance p < 0.05

DISCUSSION

The result shown in the Table 1, clearly indicate a significant increase in LPO and decrease in the protein content of the liver in CCl_4-treated liver homogenate as compared to the controls. CCl_4 has been extensively used to study liver injury induced by ROS in the mouse model, which is closely analogous to hepatotoxicity in humans. The hepatotoxic effect of CCl_4 is thought to be initiated as a result of its reductive dehalogenation by cytochrome P450 2E1 to the highly reactive trichloromethyl radical ($^{*}CCl_3$), which is subsequently converted into a trichloromethylperoxyl radical ($^{*}OOCCl_3$) in the presence of oxygen [16]. Removal of hydrogen atoms from unsaturated fatty acids by such radicals generates carbon-centred lipid radicals. These lipid radicals quickly add molecular oxygen to form lipid peroxyl radicals, thereby initiating the process of lipid peroxidation . Unless neutralized by radical scavengers, these peroxyl radicals in turn abstract hydrogen atoms from other lipid molecules, thereby propagating the process of LPO [17]. Also, $^{*}CCl_3$ can react with sulfhydryl groups, such as reduced glutathione (GSH) and protein thiols, which eventually leads to abnormal protein function, membrane LPO and, consequently, damage to mitochondria and nuclei, leading to impaired physiological functions of hepatocytes, and finally, to cell necrosis [18]. Concurrent addition of hydro-alcoholic extract of *Eclipta alba* (25-150 µg/ml) along with CCl_4 (10 µg/ml) caused concentration-dependentsignificant decrease in lipid peroxidation in liver homogenate. This may have been due to the presence of radical scavengers showing antioxidant property. Administration of CCl_4 to rats results in a wide spread dislocation of ribonucleoprotein particles from the membranes of the rough endoplasmic reticulum. This results in a depressed capacity of liver microsomes to incorporate amino acids causing a generalized

inhibition of protein synthesis. Administration of hydro-alcoholic extract of *Eclipta alba* enhanced the protein synthesizing function of the liver and increased the total protein content [19]. The antioxidants react with stable free radical DPPH (deep violet colour) and convert it to 1, 1-di phenyl-2-picryl hydrazine with decolouration. The scavenging effects of extract increased with their concentrations to similar extents. *Eclipta alba* (82.47%) showed potent DPPH radical scavenging activity (Figure 1) at the concentration of 250 µg/ml than compared to standard BHT. It could be due to various antioxidative constituents present in *Eclipta alba* [20.] Liv.52, used as a reference standard drug in present study, is a well-known hepatoprotective polyherbal formulation used in the treatment of liver diseases, evidenced by various experimental and clinical studies.

CONCLUSION

It is concluded that hydro-alcoholic extract of *Eclipta alba* effectively ameliorates the toxic effect of CCl_4- induced lipid peroxidation and protein content in liver tissue even comparatively higher than the Liv. 52, in a concentration-dependent manner. It offered significant protection to the liver and proves its antihepatotoxic effect.

ACKNOWLEDGEMENT

We thank the Gujarat University, Ahmedabad for providing laboratory facility for the study.

REFERENCES

1. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J.:Functional food science and defence against reactive oxidative species. British Journal of Nutrition; Aug;80, (1, Suppl) 1: S77–S112, (1998).
2. Medina J, Moreno-Otero R: Pathophysiological basis for antioxidant therapy in chronic liver disease.Drugs. 65(17): 2445–61, (2005).
3. Kataria M & Singh L N, Hepatoprotective effect of Liv.52 and Kumaryasava on carbon tetrachloride induced hepatic damage in rats, Indian Journal of Experimental Biology: (35), June 655-657, (1997).
4. Krithika R, Verma RJ (2009) Ameliorative potential of *Phyllanthus amarus* against carbon tetrachloride induced hepatotoxicity. Acta Poloniae Pharmaceutica - Drug Research, Vol.66 No. 5:579-583, (2009).
5. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacology & Therapeutics.;43(1):139–154, (1989).
6. Williams AT, Burk RF. Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Semin Liver Dis ;10:279–284 (1990).
7. Edwards MJ, Keller BJ, Kauffman FC, Thurman RG. The involvement of Kupffer cells in carbon tetrachloride toxicity. Toxicology and Applied Pharmacology; 119:275–279, (1993).
8. Planagumà A, Clària J, Miquel R, et al. The selective cyclooxygenase-2 inhibitor SC-236 reduces liver fibrosis by mechanisms involving non-parenchymal cell apoptosis and PPARγ activation. Faderation of American Societies of Experimental Biogy Journal.Jul;19(9):1120–1122, (2005).
9. Lamaison, JLC and Carnet A. Teneursenprincipaux flavonoids des fleurs de *Crataegeusmonogyna*Jacqet de *Crataegeuslaevigata* (Poiret D. C) en function de la vegetation. PharmaceuticaActa Helvetiae.65: 315-320, (1990).
10. Jagota SK, Dani HM. A new colorimetric technique for the estimation of vitamin C using Folin-Phenol reagent. Analytical Biochemistry 127:178-182, (1982).
11. WHO protocol CG-06.1983. APJF/IP1001 A, world health organization, Geneva, (1983).

12. Gyamfi MA, Yonamine M, Aniya Y. Free radical scavenging action of medicinal herbs from Ghana Thonningiasan guinea on experimentally induced liver injuries. General Pharmacology; 32: 661-667, (2002).
13. Ohkawa H, Ohishi N, Yagik: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358, (1979).
14. Verma RJ, Trivedi MH, Keswani H, Choksi P, &Sangai N, Ameliorative effect of three medicinal plants (*Phyllanthus fraternus, Terminalia arjuna, and Moringa oleifera*) on arsenic trioxide induced alteration of lipid peroxidation and protein contents in chicken liver: an *in vitro* study. Acta Poloniae Pharmaceutica - Drug Research,63: 417-421, (2007).
15. Lowry, OH; Rosenbrough, NJ; Farr, AL; Randall, RJ, "Protein measurement with the Folin Phenol Reagent", Journal of Biological Chemistry, 193(1):265-275, (1951).
16. Guillouzo A. Hepatotoxicity. In: Frazier JM, editor. In vitro toxicity test applications to safety evaluation. *New York: Marcel Dekker*, *Inc*. p. 45-53.
17. Bhatt AD, Bhatt NS. Indigenous Drugs and Liver Disease. Indian Journal of Gastroenterology; 15:63-67, (1996).
18. Lal VK, Amit K, Prashant K and Kuldeep SY. Screening of Leaves and Roots of *Eclipta alba* for Hepatoprotective Activity. Archives of Applied Science Research;Vol. (1): 86-89, (2010).
19. Smuckler EA, Iseri OA, Benditt EP. An intracellular defect in protein synthesis induced by carbon tetrachloride. Journal of Experimental Medicine,116, 55, (1962).
20. Nivedita and Vijay P, Physiological and phytochemical analysis of *Eclipta alba.* International journal of pharma and biosciences, 4(3):882-889, (2013).