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## DEVELOPMENT AND CHARACTERIZATION OF SOYA PHOSPHATIDYLCHOLINE COMPLEX OF COUMESTANS FROM *ECLIPTA ALBA* FOR THE EFFECTIVE MANAGEMENT OF HEPATOTOXICITY

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### Keywords:

Soya phosphatidylcholine,  
Coumestans, *Eclipta Alba*,  
Hepatotoxicity, Wedelolactone

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**ABSTRACT:** It is known that *Eclipta alba* Hassk. (Family: Compositae) contains coumestans (Wedelolactone and dimethyl wedelolactone) are used in liver disorders. The objective of the present investigation was to develop a novel formulation of these coumestans in combination with the soya phosphatidylcholine (PC), to overcome the limitation of absorption and to investigate the protective effect of the coumestans-phosphatidylcholine complex (C-PC) on carbon tetrachloride-induced acute liver damage in rats. Methanolic extract (ME) of the whole plant of *Eclipta alba* was fractionated with water and then with ethyl acetate. The ethyl acetate fraction of methanolic extract (EFME) contains coumestans which were characterized by chemical tests. EFME and PC prepared the C-PC. FT-IR and DSC spectroscopy characterized the C-PC. *In-vitro* drug release from EFME and C-PC through egg membrane was performed using UV-Visible spectrophotometer. The hepatoprotective activity of C-PC (Equivalent to 5.35 and 10.7 mg/kg body weight of EFME), ME 250 mg/kg and EFME 5.35 mg/kg were evaluated on various enzymes level. C-PC significantly provided better protection to the liver by restoring the enzyme levels of SGPT (Serum glutamate pyruvic transaminase), SGOT (Serum glutamate oxaloacetate transaminase), ALP (Alkaline phosphatase) and total bilirubin on carbon tetrachloride (CCl<sub>4</sub>) induced liver damaged animals (P<0.001). Histopathological studies were performed, and the results showed that the C-PC provided better protection to rat liver than ME and EFME at similar doses as well as shown significant regeneration of hepatocytes, central vein, intact cytoplasm and nucleus.

**INTRODUCTION:** *Eclipta alba* (L.) Hassk. (Syn. *Eclipta prostrata* L.), is a plant belonging to the family Asteraceae. It grows commonly in moist places as a weed all over the world.

It is widely distributed throughout India, China, Thailand, and Brazil. *E. alba* is widely used in India as a cholagogue and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gallbladder<sup>1</sup>. It is also reported to improve hair growth and color<sup>2</sup>. The herb *Eclipta alba* contains mainly coumestans, *i.e.* wedelolactone and demethyl-wedelolactone,  $\beta$ -amyryn, ecliptine, ecliptal,  $\alpha$ -trimethylmethanol, stigmaterol, 2- formyl-  $\alpha$ -terthenylandloteolin-7 glucoside<sup>3,4,5</sup>.

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Coumestans are being used in phytopharmaceutical formulations of medicines prescribed for the treatment of cirrhosis of the liver and infectious hepatitis<sup>6</sup>. Wedelolactone and demethyl wedelolactone both constituents exhibit antihepatotoxic activity<sup>7</sup>. Wedelolactone is also used for direct inhibition of IKK (Inhibitor of Kappa beta kinase) complex resulting in suppression of LPS (Lipopolysaccharide) -induced caspase-11 expression<sup>8</sup>, anti-venom<sup>9</sup> and antioxidant<sup>10</sup>. Most of the bioactive constituents of herbal drugs are water soluble molecules. However, water-soluble phytoconstituents like flavonoids, *i.e.*, coumestans are poorly absorbed either due to their multiple-ring large size molecules which cannot be absorbed by simple diffusion or due to their poor miscibility with oils and other lipids, severely limiting their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine<sup>11</sup>. Wedelolactone has lesser hydrophobicity<sup>12</sup>, so it may be assumed that it will have poor absorption profile.

Keeping in view, the promise of coumestan (Wedelolactone) as a therapeutically active agent and its poor oral absorption, it is pertinent to develop a new formulation of coumestan, which can increase its oral absorption and enhance its therapeutic activity. Several studies have indicated the beneficial role of phospholipids in enhancing the therapeutic efficacy of some molecules having poor oral absorption. Silybin is one such molecule having poor oral bioavailability. Efforts have been given to prepare phospholipids complex of silybin and to increase its oral bioavailability, therefore, therapeutic efficacy. It was observed that the silybin-phospholipids complex has the significant upper hand over the pure molecule in protecting the liver and exerting antioxidant activities<sup>13, 14, 15, 16, 17</sup>. A recent study with quercetin-phospholipid complex showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride<sup>18</sup>.

Aim of the present study was to prepare phospholipids complex of coumestans (*i.e.* wedelolactone) to determine its physicochemical properties and its hepatoprotective activity in carbon tetrachloride intoxicated rats in comparison to ME and its ethyl acetate fraction (EFME), to substantiate the claim that complexation between

coumestan (Wedelolactone) and phospholipid can enhance the therapeutic efficacy of the parent molecule by increasing its absorption.

#### EXPERIMENTAL:

**Materials:** *Eclipta alba* was collected from the area of Shahgarh District, Sagar (M.P.), India and authenticated by Dr. Amarjeet Bazaz, MVM College (reference number 01/2008), Bhopal, India. Phospholipid hydrogenated soy phosphatidylcholine (PC) was purchased from Lipoid, Ludwigshafen, Germany. Wedelolactone was received as a gift sample from Laila Impex Vijayawada, India. Dichloromethane, *n*-hexane, and chloroform were purchased from Rankem (Pvt.) Ltd. Assay kits for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total bilirubin and alkaline phosphatase (ALP) were purchased from vital diagnostics Ltd., Mumbai, India. Animals used in the study were obtained from animal house of VNS Institute of Pharmacy, Bhopal, India and were kept under pathogen-free conditions. All other reagents used were of analytical grade and were used as received.

**Extraction and Fractionation:** The dried powdered plant material (463 g) was extracted with methanol (3.0 L) by maceration with agitation at room temperature for 24 h. The solvent was removed with reduced pressure obtaining a dark green residue (90.3 g). Part of the residue (70 g) was suspended in 770 ml of water and warmed in a water bath at <75 °C for 30 min, the aqueous phase was filtered and successively extracted four times with 250 ml of ethyl acetate; the ethyl acetate fraction (brown) was dried under vacuum giving 1.50 g of brown residue. Legal test and Keddie's test were performed to confirm the presence of lactones in the EFME. TLC studies on EFME and standard drug wedelolactone were performed using stationary phase silica gel 60 F<sub>254</sub> using mobile phase, CHCl<sub>3</sub> + Methanol (70:30), FeCl<sub>3</sub> 0.5N was used as detecting agent<sup>7</sup>.

#### Preparation of Coumestan - Phospholipid

**Complex:** The complex was prepared with EFME and PC at a ratio of 1:1. A weighed amount of EFME and PC were taken in a 100 ml round bottom flask and 50 ml of dichloromethane were added. The mixture was agitated continuously on a magnetic stirrer at a temperature not exceeding

40°C for 2 h. The resultant clear solution was concentrated in vacuum to a 1/3 of its volume, and 25 ml of *n*-hexane was added to it with continuous stirring. The C-PC was precipitated, and the precipitate was filtered and dried under vacuum to remove traces of solvents.

**Solubility Studies:** EFME, phosphatidylcholine and the prepared C-PC 10 mg were dissolved in 2 ml chloroform separately to check the solubility profile.

**Microscopic View of the Complex:** The complex was suspended in distilled water, and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 400X (Labomed microscope type CX R3).

**FT-IR Spectroscopy:** FT-IR spectra for the various powders and semisolids of EFME, PC, PM, and C-PC were obtained on an 8400 Shimadzu FTIR spectrometer (Shimadzu, Tokyo, Japan) in the transmission mode with the wave number region 500-4,000  $\text{cm}^{-1}$ . KBr pellets were prepared by gently mixing 1 mg sample powder with 100 mg KBr.

**Differential Scanning Calorimetry (DSC):** Thermograms of EFME, PC, C-PC and PM (1:1 ratio) of EFME and PC, were recorded using Mettler Toledo Star\* system (UK). The samples were sealed in the aluminum crimp cell and heated at the speed of 10 °C/min from 10 to 350 °C in the nitrogen atmosphere (60 ml/min).

**Dissolution Study:** The dissolution study was performed to determine the effect of complex formation on the release profile of wedelolactone. For that purpose egg membrane was selected because egg membrane mimics the biological membrane and *in-vivo* performance based on *in-vitro* results can be predicted more accurately. The release through egg membrane was measured for 2 h. Samples were withdrawn and replaced with fresh media to maintain a constant volume of dissolution media. Concentration measurement was done spectrophotometrically using UV-Visible spectrophotometer at 347 nm.

**In-vivo Studies:** All experiments were performed due permission from the Institutional Animal

Ethical Committee (Reg. No. 778/03/ c/CPCSEA). Adult Albino rats (80-110 g) of either sex were chosen for hepatoprotective activity. All the tests were carried out in a quiet laboratory setting with ambient illumination and at temperature  $37 \pm 2$  °C. Animals were allowed to acclimatize to the laboratory for 7 days before the experiment begins. Animals were divided into six groups of six in each. Group 1 served as control and received vehicle 1% w/v gum tragacanth at a dose of 1 ml/kg p.o. for 4 days and coconut oil (1 ml/kg i.p.) on day 2 and 3. Groups 2, 3, 4, 5 and 6 received 2 ml/kg i.p. of  $\text{CCl}_4$ : coconut oil (1:1) on second and third day 30 min after treatment with ME, EFME and C-PC. Animals from  $\text{CCl}_4$  group received vehicle 1% w/v gum tragacanth at a dose of 1 ml/kg p.o. for 4 days. The ME of *Eclipta alba* was administered to Group 3 at the dose of 250 mg/kg p.o. for 4 days. The EFME of *Eclipta alba* was administered to Group 4 at the dose of 5.35 mg/kg p.o. for 4 days. Groups 5 and 6 were treated with C-PC at doses equivalent to 5.35 mg/kg p.o. and 10.7 mg/kg p.o. of EFME for 4 days, respectively. The  $\text{CCl}_4$ , ME, EFME and C-PC were administered concomitantly to the respective groups of animals. All the animals were sacrificed on day 5 under light ether anesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37 °C. The clear serum was separated at 2500 rpm for 10 min, and biochemical parameters were evaluated to assess liver functions *viz.*, total bilirubin<sup>19</sup>, SGPT<sup>20</sup>, SGOT<sup>21</sup> and serum ALP<sup>22</sup> using star 21plus autoanalyzer. The results were summarized in **Table 1**.

**Statistical Analysis:** All the experiments were conducted using at least six animals in each group, and the biochemical estimations have been reported as mean  $\pm$  SEM and calculated for each parameter. The statistical significance between groups was analyzed using one way ANOVA followed by Dunnett's Multiple Comparison Test. A P-value ( $<0.001$ ) was considered significant.

**Histopathology:** A portion of liver tissue in each group was fixed in 10% formalin then after paraffin embedding and block making, serial sections of 5m thicknesses were made, stained with hematoxylin and eosin and examined under Labomed microscope CX R3.

## RESULTS AND DISCUSSION:

**Extraction and Fractionation:** For fractionation, the residue of the ME of the whole plant of *Eclipta alba* was first treated with water, and the aqueous phase was fractionated with ethyl acetate. Legal test and Keddie's test were found positive showing presence of lactones in the EFME. TLC studies have shown the presence of wedelolactone in the EFME ( $R_f$  value 0.61).

**The yield of Prepared Complex:** C-PC was prepared by a simple and reproducible method, yielding  $70.0 \pm 2.26\%$  of the product. The resultant C-PC was kept in an amber colored glass bottle, stored at room temperature.

**Solubility Profile:** Solubility test revealed that EFME was not soluble in chloroform, while PC and the prepared C-PC were soluble indicating that a reaction occurred between the EFME and PC resulting in producing a lipophilic envelope which allows the complexed coumestans to dissolve in low polarity solvents.

**Microscopic Observations:** The microscopic view **Fig. 1** shows the presence of spherical structures of the C-PC. The vesicles consisted of PC, and coumestans were intercalated in the lipid layer.

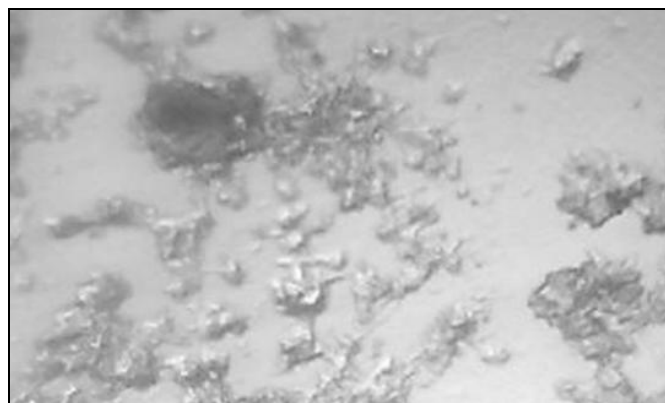


FIG. 1: PHOTOMICROGRAPH OF C-PC AT 400X

**FT-IR Spectroscopy:** FT-IR studies were done to detect the possible interaction between EFME and PC in the C-PC **Fig 2A, 2B** and **2C**.

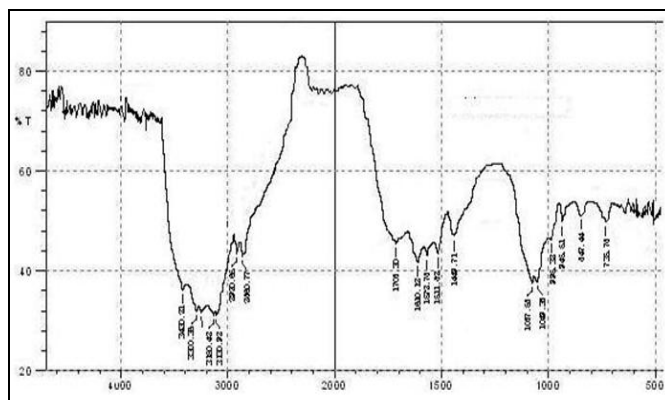


FIG. 2A: IR SPECTRA OF EFME

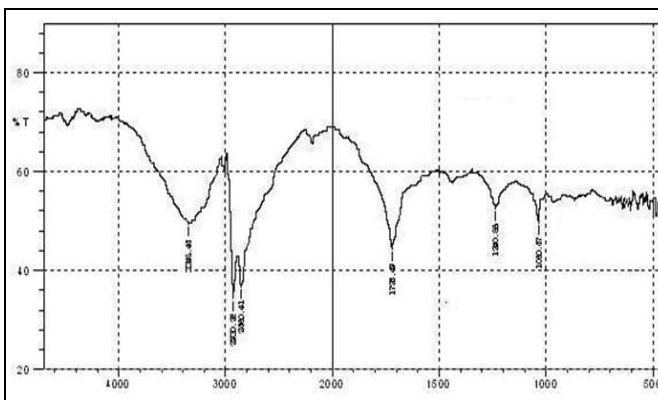


FIG. 2B: IR SPECTRA UNPROCESSED PC

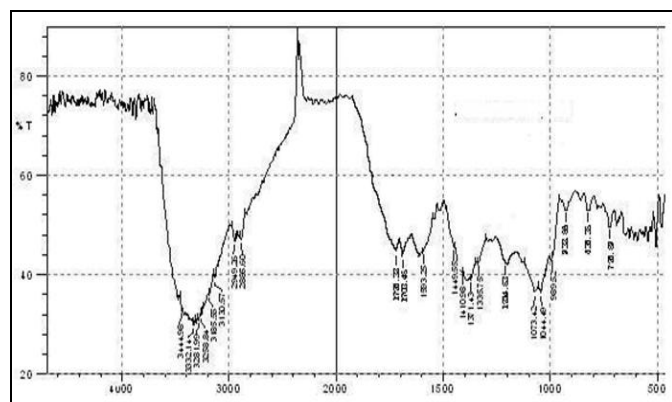


FIG. 2C: IR SPECTRA OF C-PC

Hydroxyl ( $-\text{OH}$ ) stretching band at  $3345.48 \text{ cm}^{-1}$ ,  $-\text{C}-\text{H}$  stretching band of saturated long fatty acid chain at  $2930.26$  and  $2860.77 \text{ cm}^{-1}$ , carbonyl

stretching band at  $1728.49 \text{ cm}^{-1}$  in the fatty acid ester,  $-\text{P}=\text{O}$  stretching band at  $1240.56 \text{ cm}^{-1}$  and  $-\text{P}-\text{O}-\text{C}$  stretching band at  $1060.87 \text{ cm}^{-1}$  in the PC structure, whereas vibration peaks of  $-\text{OH}$  stretching ( $n\text{OH}$ ,  $3300.36 \text{ cm}^{-1}$ ),  $-\text{C}=\text{O}$  ( $n\text{C}=\text{O}$ ,  $1708.30 \text{ cm}^{-1}$ ),  $-\text{C}=\text{C}$  vibration in the benzene-ring ( $n\text{C}=\text{C}$ ,  $1610.12$ ,  $1572.78$ ,  $1511.82$ ,  $1449.71 \text{ cm}^{-1}$ ),  $-\text{C}-\text{O}-\text{R}$  ( $n\text{C}-\text{O}$ ,  $1049.38 \text{ cm}^{-1}$ ) and the  $-\text{C}-\text{C}$  single bond ( $n\text{C}-\text{C}(\text{benzene})$ ,  $945.51$ ,  $847.44$ ,  $735.78 \text{ cm}^{-1}$ ) were detected in the IR spectra of EFME. The spectra of the C-PC shows shifts, disappearances or attenuation of the characteristic EFME bands revealed a modification of its environment, hence providing evidence for the possible interaction occurring between the EFME

and PC. The C-PC spectra exhibited a shift in the benzene-ring framework stretching bands from 1610.12 and 1572.78  $\text{cm}^{-1}$  to a new band at 1593.25  $\text{cm}^{-1}$ , and  $\text{-C-C}$  vibrations of benzene-ring moved to lower wave numbers of 932.56, 833.2 and 728.59  $\text{cm}^{-1}$ , all suggesting a possible change in the environment of benzene-ring framework of coumestans present in EFME.

### Differential Scanning Calorimetry (DSC):

Differential scanning calorimetry (DSC) represents a thermoanalytical method that measures the

difference in the thermal energy required to increase the temperature of a sample and a reference with well-defined heat capacity as a function of the temperature. Ideally, both the sample and the reference are kept at an identical temperature, while the temperature increases linearly as a function of time. As soon as the sample undergoes a physical transformation; either exothermic or endothermic, the heat flow to the sample will be decreased or increased relative to the reference, thus, resulting in a DSC signal<sup>23</sup>.

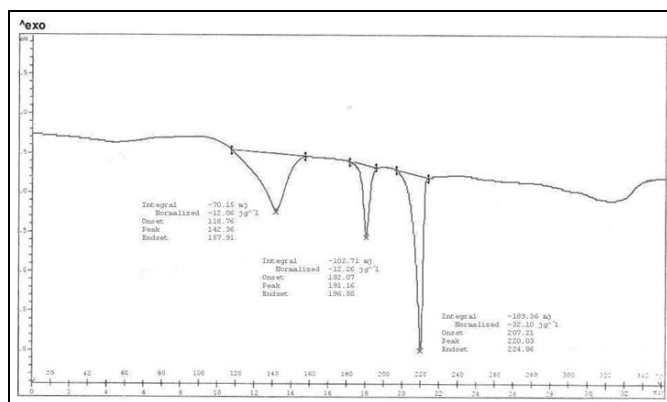


FIG. 3A: DSC THERMOGRAM OF EFME

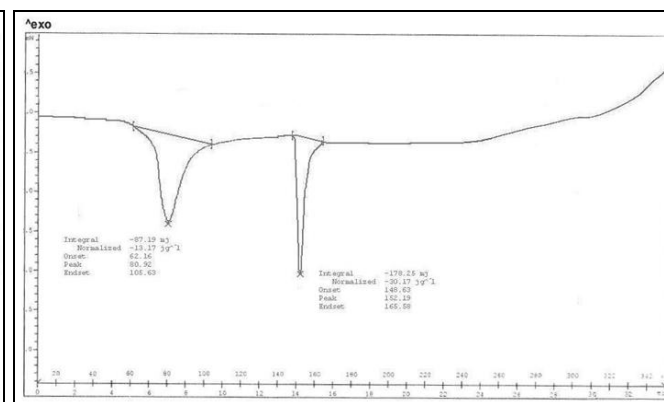


FIG. 3B: DSC THERMOGRAM UNPROCESSED PC

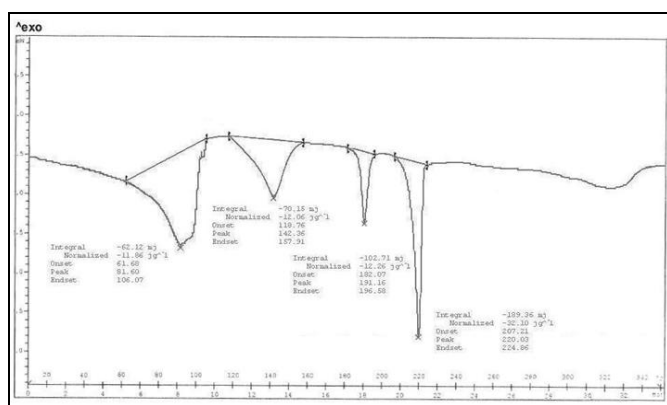


FIG. 3C: DSC THERMOGRAM OF PM

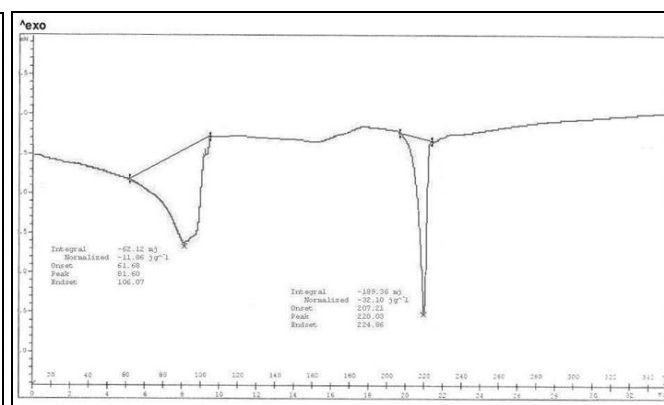


FIG. 3D: DSC THERMOGRAM OF C-PC

Fig. 3A, 3B, 3C and 3D shows the DSC thermograms of EFME (3A), PC (3B), C-PC (3C) and physical mixture (PM) of EFME and PC (3D). The thermogram of EFME shows three peaks first broad at 142.36 °C, second at 191.16 °C and third sharp at 220.03 °C. Thermogram of PC exhibit two different peaks; the first peak (80.92 °C) is mild, which appears because of the hot movement of PC polar head group. The second peak (152.19 °C) is very sharp, and it appears due to phase transition from gel to the liquid crystalline state. The non-polar hydrocarbon tail of PC may be melted during this phase, yielding a sharp peak. Two peaks were

appeared in the DSC thermogram of C-PC first broad peak, with an onset at 61.68 °C and maximum occurrence at 106.07 °C, which is different from the peaks of the individual components of the complex and second sharp at 220.03 °C also present in the DSC curve of EFME. PM of EFME and PC shows four peaks, first at 81.60 °C, second at 142.36 °C third at 191.16 °C and fourth at 220.03 °C. The first has the same onset temperature of 61.68 °C of the C-PC, and the later three have same onset temperatures 118.76, 182.07 and 207.21 °C of EFME. It may be assumed that with the rise in temperature the PC become

melted and EFME gets dissolved in PC, partly forming the complex. The thermogram of the C-PC exhibits a peak which differs from the peak of EFME and PC. It is evident that the original peaks of EFME and PC disappear from the thermogram of C-PC and the phase transition temperature is lower than that of PC.

**Dissolution Study:** Comparative dissolution study was performed between EFME of *Eclipta alba* and C-PC. The data are shown in Fig. 4. The less quantity of drug detected in case of EFME which may be attributed to hydrophilic nature of the wedelolactone, which prevent it from crossing through the egg membrane while higher absorbance was detected in case of C-PC. The higher concentration in case of C-PC may be attributed due to the presence of a PC, which provides lipophilicity to the resulting complex. The lipophilicity makes it possible to easily cross through the egg membrane. From the data, it may be hypothesized that the C-PC will show better bioavailability in comparison to EFME.

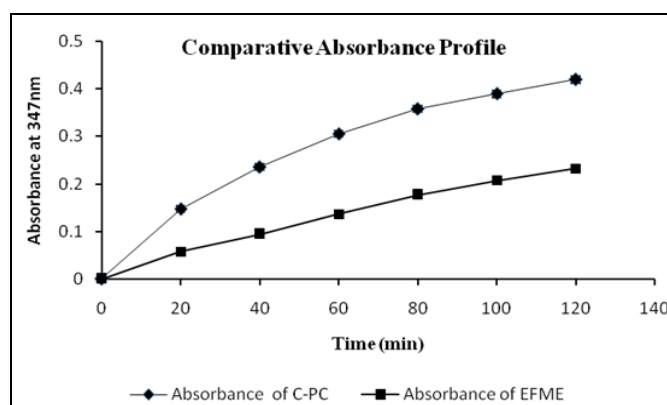


FIG. 4: COMPARATIVE ABSORBANCE PROFILE OF C-PC AND EFME

**In-vivo Studies:** Since, the changes associated with  $\text{CCl}_4$ -induced liver damage are similar to that of acute viral hepatitis<sup>24</sup>. The hepatotoxicity induced by  $\text{CCl}_4$  is due to its metabolite  $\text{CCl}_3\cdot$  carbon trichloride free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on lipids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage<sup>25</sup>.

TABLE: 2 EFFECT OF ME, EFME AND C-PC OF *ECLIPTA ALBA* ON  $\text{CCl}_4$ -INDUCED HEPATOTOXICITY IN RAT

Groups (n)	Dose	SGPT (U/l)	SGOT (U/l)	Bilirubin (mg/dl)	ALP (U/l)
Group 1 (Control)	1 % w/v Tragacanth suspension 1 ml/kg p.o.	44.62±0.91	75.60±1.25	0.54±0.01	178.40±3.75
Group 2 ( $\text{CCl}_4$ control)	$\text{CCl}_4$ 2 ml/kg i.p.	186.60±2.02a*	138.50±1.66a*	2.17±0.02a*	412.20±3.18a*
Group 3 (ME + $\text{CCl}_4$ treated)	250 mg/kg p.o.+ $\text{CCl}_4$ 2 ml/kg i.p.	122.40±0.94b*	109.70±1.27b*	1.42±0.01b*	289.90±2.27b*
Group 4 (EFME + $\text{CCl}_4$ treated)	5.35 mg/kg p.o.+ $\text{CCl}_4$ 2 ml/kg i.p.	95.08±1.15b*	100.60±1.3b*	1.22±0.02b*	247.90±2.35b*
Group 5 (C-PC 1 + $\text{CCl}_4$ treated)	5.35 mg/kg p.o.+ $\text{CCl}_4$ 2 ml/kg i.p.	82.86±1.37b*	94.06±1.04b*	1.07±0.02b*	217.80±1.79b*
Group 6 (C-PC 2 + $\text{CCl}_4$ treated)	10.7 mg/kg p.o.+ $\text{CCl}_4$ 2 ml/kg i.p.	69.77±0.83b*	83.47±0.95b*	0.95±0.01b*	196.30±2.13b*

Values are Mean ± SEM of 6 animals each in a group. A significant statistical test for comparison was done by ANOVA, followed by Dunnet's 't' test (n = 6). Comparison between: a-Group 1 vs. Group 2, b-Group 2 vs. Group 3, 4, 5 and 6. \*P<0.0001

Aminotransferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine aminotransferase and aspartate aminotransferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the bloodstream<sup>26</sup>. Alkaline phosphatase is a membrane-bound enzyme, and its elevations in plasma indicate membrane disruption in the organ. Alkaline phosphatases, although not a liver-specific enzyme, the liver is the major source of this

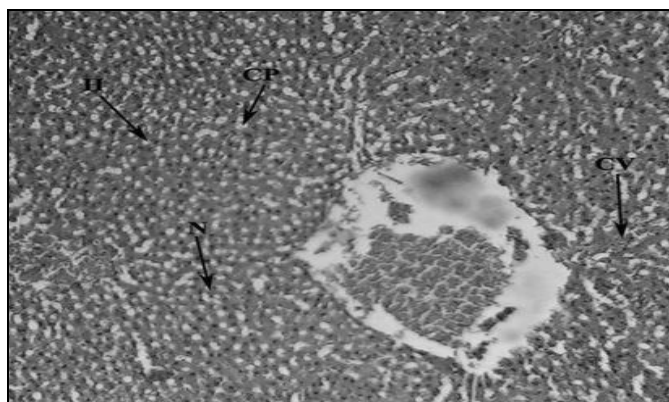
enzyme. The level of this enzyme increases in cholestasis<sup>27</sup>. Hepatotoxicity is characterized by a cirrhotic liver condition which in turn increased the bilirubin release<sup>28</sup>. Serum SGPT, SGOT, total bilirubin, and ALP levels were selected as parameters to measure the hepatoprotective effect of C-PC. Table 1 compares the effect of ME, EFME and C-PC at different dose levels on the  $\text{CCl}_4$ -induced rise in serum SGPT, SGOT, total bilirubin and ALP levels of the rat. From the results, it can be observed that group 2 was found to possess highly significant increases in (P<0.001) levels of serum SGPT, SGOT, total bilirubin, and ALP when compared with group 1.

The treatment of animals with ME at a dose of 250 mg/kg (group-3) and EFME at 5.35 mg/kg (group-4), afforded a highly significant ( $P < 0.001$ ) protection against the  $\text{CCl}_4$  induced enhanced serum SGPT, SGOT, total bilirubin, and ALP levels and the results were comparably better in EFME. Treatment of animals with C-PC equivalent to 5.35 mg/kg of EFME (group 5) provided highly significant protection ( $P < 0.001$ ) against  $\text{CCl}_4$ -induced rise in serum SGPT, SGOT, total bilirubin, and ALP levels. The results were comparably better in comparison with group 3 and group 4.

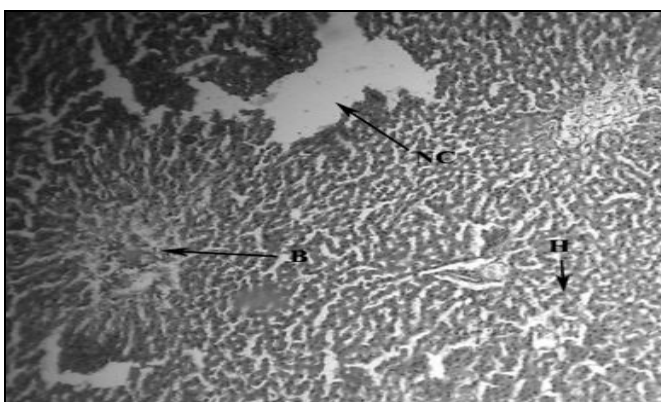
On comparing the SGPT, SGOT, total bilirubin, and ALP levels, it was observed that C-PC at the dose equivalent to 10.7 mg/kg of EFME (group 6) revealed significantly greater protection against  $\text{CCl}_4$ -induced serum SGPT, SGOT, total bilirubin, and ALP levels. The results were better as compared to C-PC at the dose equivalent to 5.35 mg/kg of EFME (group 5). Thus, a dose-dependent hepatoprotective activity of C-PC was observed.

**Histopathological Observations:** Histopathological studies of liver were observed for a visual assessment of hepatic architecture. **Fig. 5A, 5B, 5C, 5D, 5E, and 5F** show the representative photomicrographs of a liver section of rats. The rat treated with vehicle control (group 1) **Fig. 5A** showed a normal hepatic architecture as hepatocytes, central vein, nucleus, cytoplasm, and visible portal tract.

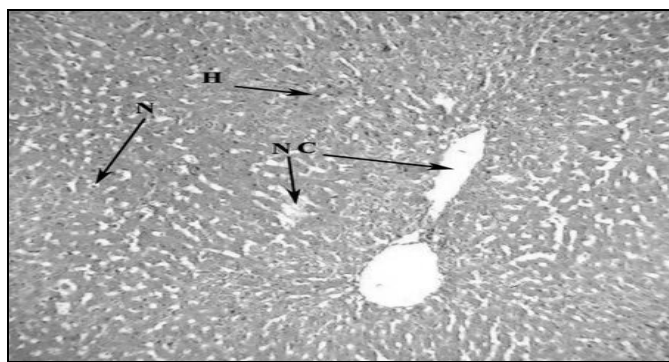
Treatment with  $\text{CCl}_4$  (2 ml/kg) (group 2) resulted in acute hepatotoxicity as observed from massive fatty changes, necrosis, ballooning degeneration, degeneration of hepatocytes and loss of cellular boundaries **Fig. 5B**. Treatment of animals with ME (250 mg/kg, group 3) resulted in hepatoprotection, as can be observed by the recovery of hepatocytes, lesser necrosis and a well-identified nucleus which indicate normal hepatic architecture **Fig. 5C**. The similar results were observed on pretreatment of animals with EFME at 5.35 mg/kg (group 4) **Fig. 5d**.



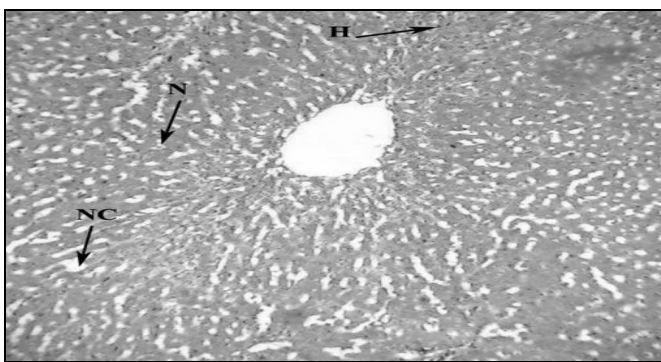
**FIG. 5A:** LIVER SECTIONS OF NORMAL SHOWING NORMAL HEPATIC CELLS (H) WITH WELL PRESERVED CYTOPLASM (CP); WITH WELL BROUGHT OUT CENTRAL VEIN (CV); PROMINENT NUCLEUS (N) AND NUCLEOLUS



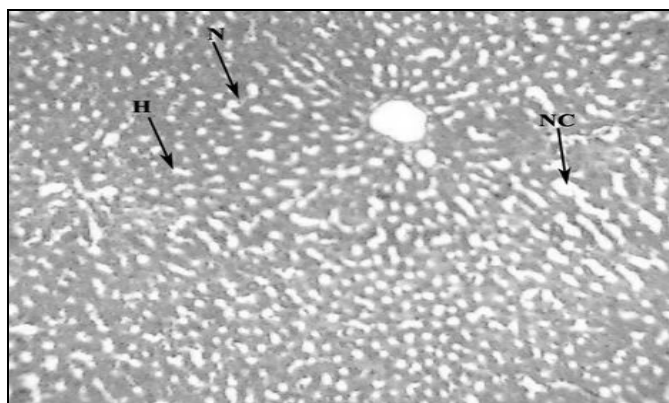
**FIG. 5B:** LIVER SECTIONS OF CONTROL SHOWING: MASSIVE FATTY CHANGES, NECROSIS (NC), BALLOONING DEGENERATION (B), DEGENERATION OF HEPATOCYTES, HEPATOCYTES (H) AND LOSS OF CELLULAR BOUNDARIES



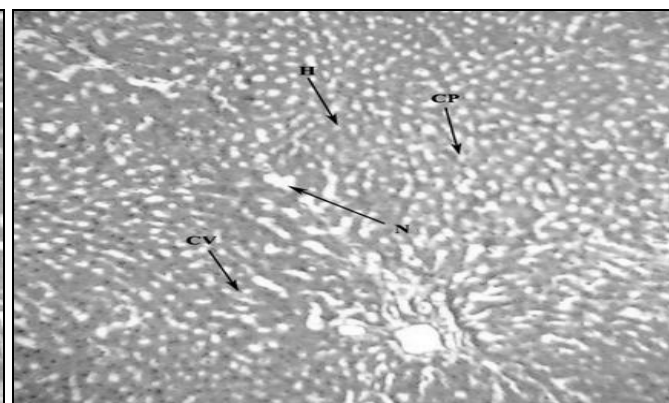
**FIG. 5C:** LIVER SECTIONS OF RATS TREATED WITH ME AND  $\text{CCl}_4$  SHOWING: RECOVERY OF HEPATOCYTES (H), LESSER NECROSIS (NC) AND WELL IDENTIFIED NUCLEUS (N)



**FIG. 5D:** LIVER SECTIONS OF RATS TREATED WITH EFME AND  $\text{CCl}_4$  SHOWING: RECOVERY OF HEPATOCYTES (H), LESSER NECROSIS (NC) AND WELL IDENTIFIED NUCLEUS (N)



**FIG. 5E: LIVER SECTIONS OF RATS TREATED WITH C-PC AT DOSE EQUIVALENT TO 5.35 mg/kg OF EFME AND CCl<sub>4</sub> SHOWING: RECOVERY OF HEPATOCYTES (H), LESSER NECROSIS (NC) AND WELL IDENTIFIED NUCLEUS (N)**



**FIG. 5F: LIVER SECTIONS OF RATS TREATED WITH C-PC AT DOSE EQUIVALENT TO 5.35 mg/kg OF EFME AND CCl<sub>4</sub> SHOWING: RECOVERY OF HEPATOCYTES (H), CENTRAL VEIN (CV), WELL PRESERVED CYTOPLASM (CP) AND WELL IDENTIFIED NUCLEUS (N)**

C-PC at a dose equivalent to 5.35 mg/kg and 10.7 mg/kg of EFME (group 5 and 6) showed better protection than EFME as observed by the presence of normal hepatocytes, central vein, intact cytoplasm and nucleus **Fig. 5E and 5E**.

**CONCLUSION:** The hepatoprotective activity of ME 250 mg/kg, EFME 5.35 mg/kg and C-PC (equivalent to 5.35 and 10.7 mg/kg body weight of EFME) was evaluated by measuring SGPT, SGOT, Bilirubin, and ALP enzymes level. From the results, it can be observed that animals of CCl<sub>4</sub> control were found to possess highly significant increases in levels of serum SGPT, SGOT, total bilirubin, and ALP when compared with normal. The treatment of animals with ME at a dose of 250 mg/kg and EFME at 5.35 mg/kg and C-PC equivalent to 5.35 mg/kg of EFME afforded highly significant protection against the CCl<sub>4</sub> induced enhanced serum SGPT, SGOT, total bilirubin, and ALP levels.

The results were comparably better in C-PC than EFME and ME and ME was found least active. The treatment of animals with C-PC at the dose equivalent to 10.7 mg/kg of EFME revealed significantly greater protection against CCl<sub>4</sub>-induced serum SGPT, SGOT, total bilirubin and ALP levels. The results were better as compared to C-PC at the dose equivalent to 5.35 mg/kg of EFME. Thus, a dose dependent hepatoprotective activity of C-PC was observed. Histopathological studies were also performed. The rat treated with a vehicle control showed a normal hepatic architecture as hepatocytes, central vein, nucleus, cytoplasm and visible portal tract.

Treatment with CCl<sub>4</sub> (2 ml/kg) resulted in acute hepatotoxicity as observed from massive fatty changes, necrosis, ballooning degeneration, degeneration of hepatocytes and loss of cellular boundaries. Treatment of animals with ME 250 mg/kg resulted in hepatoprotection, as can be observed by the recovery of hepatocytes, lesser necrosis and a well identified nucleus which indicate normal hepatic architecture. The similar results were observed on pre treatment of animals with EFME at 5.35 mg/kg. C-PC at a dose equivalent to 5.35 mg/kg and 10.7 mg/kg of showed better protection than EFME as observed by the presence of normal hepatocytes, central vein, intact cytoplasm and nucleus. These findings suggested that ME, EFME and C-PC administration has significantly neutralized the toxic effect of CCl<sub>4</sub> and helped in the regeneration of hepatocytes. The investigations also confirm that the phosphatidylcholine complex is another breakthrough in the development of herbal products as the complex formation process enhances the efficacy of herbal compounds by improving their absorption, bioavailability, and delivery of therapeutic substances of herbal origin to the specific tissue or organ involved.

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