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# Evaluation of Hepatoprotective And Antihepatotoxic Activity of Ethanolic Extract of *Evolvulus alsinoides* Linn on $CCL_4$ Induced Rats

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## ABSTRACT

Liver is the largest organ of the body, it plays vital role in regulation of body functions. Various herbal medicines were used traditionally for liver disorders. In this study we evaluated hepatoprotective activity and antihepatotoxic activity of ethanolic extract of *Evolvulus alsinoides* Linn(EAEE) was aganist CCl4 induced hepatotoxicity model in albino Wistar rats (150 – 200 gms) at a dose of 75 & 150 mg/kg b.w p.o. It was reported that *Evolvulus alsinoides* was used in the treatment of hypertension, memory enhancer, stomachic, nervous disorders etc. Preliminary phytochemical studies of the EAEE showed the presence of Carbohydrates, Flavonoids, Glycoside, Alkaloids and Proteins. There was no mortality when conducting the acute toxicity studies, upto a dose of 1500 mg/kg b.w p.o. Hence two test doses of 75 and 150 mg/kg b.w p.o were selected in the study. Hepatotoxicity was induced with CCl4, silymarin 50 mg/kg b.w.p.o. was used as standard. Administration of CCl4, significantly elevated the levels of SGPT, SGOT, ALP, TB, CHOL and lowered the levels of TP and ALB. When EAEE 150 mg/kg produced significant inhibition of hepatic damage by significantly ($P<0.01$) reversing the effects of CCl4 induced hepatotoxicity. Histopatholigical studies revealed that 90% Ethanolic extract exhibited normalization of liver architecture, as compared to silymarin. Results of our study suggest that Ethanolic extract of *Evolvulus alsinoides* may possess hepatoprotective activity which may be due to the presence of flavonoids in the extract.

**Keywords:** *Evolvulus alsinoides*, Silymarin, Pentobarbitone sodium, Ethanol.

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## 1. INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. The liver, because of its strategic anatomical location, is exposed to many kinds of xenobiotics and therapeutic agents. Moreover, the rapidly increasing morbidity and mortality rates from liver diseases are largely attributable to the repeated chemical insult either from drug abuse or from environmental pollution. Unfortunately so far, in the modern era of medicine there is no specific treatment to counter the life threatening impact of these dreaded conditions[1,2]

Herbal-based therapeutics for liver disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. Despite the significant popularity of several herbal medicines in general, and for liver diseases in particular, they are still unacceptable treatment modalities for liver diseases.

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines[3].

*Evolvulus alsinoides* Linn (Convulvulaceae)[4], whole plant is widely distributed in tropical and subtropical regions throughout the world. It grows commonly as a weed in open and grassy places throughout India, ascending at 6000ft. Traditionally this plant is used in many ways like, the whole herb is used medicinally in the form of decoction with cumin and milk in fever, nervous debility, loss of memory and syphilis, decoction with *Ocimum sanctum* are is used in malarial fever, leaves are made into cigarettes and smoked in chronic bronchitis and asthma, oil promotes the growth of hair[5]. The herb is used to treat dysentery[6]. Mohammedan physicians used the plant as a general tonic to strengthen the brain and memory[7]. The plant is used to treat bowel problems and to promote conception[8,9]. The entire plant was useful for treating haemorrhages, and also variety of other medical applications, including use as an adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, stomachic, tonic, and vermifuge, in the treatment of asthma, bronchitis, scrofula, syphilis, or in "controlling night emissions," and to promote wound healing[10,11,12].

The plant contains alkaloids: betaine, shankhapushpine and evolvine. Fresh plant contains volatile oil. It also contains a yellow neutral fat, an organic acid and saline substances. An unidentified compound has been isolated. Scopoletin, scopolin, umbelliferone, 2-methyl-1,2,3,4-butanetetrol, ferulic acid esters with alcohols C14-C17 and palmitic, stearic, oleic, 8-methyldecanoic and heptadecanoic acids have been reported. 2,3,4-trihydroxy-3-methylbutyl 3-[3-hydroxy-4-(2,3,4-trihydroxy-2-methylbutoxy)-phenyl]-2-propenoate and 1,3-di-*O*-caffeoyl quinic acid methyl ester, caffeic acid, 6-methoxy-7-*O*-ß-glucopyranoside coumarin, 2-C-methyl erythritol,kaempferol-7-O-ß-glucopyranoside,kaempferol-3-O-ß-glucopyranoside and quecetine-3-O-ß-glucopyranoside were reported from *n*-BuOH soluble fraction from the ethanol extract of *E. alsinoides*[13].

Based on its uses in traditional system of medicine the present study was aimed at investigating the hepatoprotective and anti-hepatotoixic activity of ethanolic extract of *Evolvulus alsinoides* Linn (EAEE) in $CCl_4$ induced hepatotoxicity model in Albino Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant material

*Evolvulus alsinoides* whole plant were collected from the field of Thirumala hills and was authenticated by Dr. K. Madhava chetty, Professor, Department of Botany, Sri Venkateswara University, Thirupathi, Andhra Pradesh, India.

### 2.2. Preparation of Extract

The whole plant material was dried and freed from foreign material, cut in to small pieces and powdered mechanically by using soxhlet apparatus, the solvent was removed, typically by mean of a rotary evaporator, the concentrated product was used as ethanolic extract of *Evolvulus alsinoides* coded as EAEE. The obtained extracts were kept in a desiccator to remove moisture and stored properly until used.

### 2.3. Chemicals

Silymarin was obtained from Micro Labs. Ltd. Hosur, Bangalore. Carbon tetra chloride, Ethanol, Tween-80 from S.D Fine Chemicals, Mumbai. Olive oil was obtained from Seven Ships, Hyderabad. Analytical kits like Serum Glutamic Oxaloacetate Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline phosphate (ALP) Total Bilirubin (TB), Total protein (TP) and Albumin (ALB) were purchased from Span, Diagnostics Ltd., Surat, India. Colorimeter - Merck Specialties Pvt. Ltd, Mumbai. UV-Visible Spectrophotometer-Elico SL149; Centrifuge (R8-C Laboratory centrifuge)-Remi were used for the study.

**Software Used:** Graph Pad Prism ( Version 5).

### 2.4. Experimental Animals:

Healthy Wistar albino rats, weighing 150-200 gm and albino mice (15-25 gm) were procured from the Teena Biolabs Pvt. Ltd. (Reg. No. 177/99 CPCSEA), Hyderabad, Andhra Pradesh. Animals were housed at

CPCSEA approved animal house (1533/PO/a/11/CPCSEA) with light and dark cycle and had free access to commercial pellet diet (Vyas labs Ltd, Hyderabad, India) with water *ad libitum.* The animal house temperature was maintained at 25 ± 2 $^0$C with relative humidity at (50 ±15%). The study strictly followed Ethical norms during all experimental procedure.

**2.5. Acute Toxicity Studies**

The acute oral toxicity procedure was followed by using OECD 423 guidelines. Healthy Wistar albino mice of 20-30 g either sex were selected for the study[14]. The ethanolic extracts of *Evolvulus alsinoides* (EAEE) were suspended 1% w/v tween-80 in distilled water. EAEE was administered orally to albino mice. After the oral administration animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention in autonomic or behavioral responses during the first 4 hours and later at 24 hrs intervals for a period of 48 hrs. At the end of this period, the mortality if any, in different dose groups were noted. It was observed that the EAEE did caused mortality even at 1500 mg/kg dose. Hence the $LD_{50}$ of the extracts was found to, 1/10th (150 mg/kg), 1/20$^{th}$ (75 mg/kg) of these doses were selected for further studies.

**2.6. Pharmacological Studies:**

**2.6.1. Preventive (Hepatoprotective) Studies**[14]

In the present study, the animals were pretreated with test extracts before inducing liver damage with $CCl_4$. Seven days after acclimatization, the rats were divided into five groups, each group consisting of six animals. All animals were kept on same diet for 7 days.

**Group-I** served as a control and received 1ml/kg of 1 %w/v tween-80 in distilled water p.o. for seven days.

**Group-II** Treated with vehicle (1 ml/kg of 1% w/v tween-80 in distilled water p.o.) daily for seven days followed by 1 ml/kg of 50% v/v $CCl_4$ in olive oil p.o. on the seventh day.

**Group-III** served as standard animals were administered with Silymarin 50 mg/kg p.o. for seven days followed by 1 ml/kg of 50% v/v $CCl_4$ in olive oil administered p.o. on the seventh day.

**Group IV- V** test groups were treated in the similar way using ethanolic extract of *Evolvulus alsinoides* (EAEE) 75, 150 mg/kg, respectively followed by $CCl_4$ administered p.o on the seventh day.

All the rats were anaesthetized with thiopental sodium (60 mg/kg i.p), 36 hrs after administration of $CCl_4$, blood was collected from the retro-orbital plexus of rats. After blood collection, the blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analyzed for SGPT, SGOT, ALP, TB, CHOL, TP and ALB levels. The animals were then dissected, the liver were carefully removed, washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies.

$$\text{Percentage protection} = \left\{1 - \frac{T - V}{C - V}\right\} \times 100$$

Where "T" is the mean value of drug and $CCl_4$, "C" is the mean value of $CCl_4$ alone and "V" is the mean value of vehicle treated animals.

**2.6.2. Curative (Antihepatotoxic) Studies**[15]

The animals were divided into five groups (I-V) each consisting of six animals. All the animals were kept on same diet for 7 days. The animals were first treated with $CCl_4$ for inducing liver damage and then treated with extracts under investigation.

**Group-I** served as a control and received 1ml/kg of 1 %w/v tween-80 in distilled water p.o. on 1, 3 and 5$^{th}$ day.

**Group-II** served as toxic and given 1 ml/kg of 50% v/v $CCl_4$ in olive oil p.o. on 1, 3 and 5$^{th}$ day.

**Group-III** served as standard animals were administered with $CCl_4$ p.o. on 1, 3 and 5$^{th}$ day, from 6$^{th}$ to10$^{th}$ i.e. for 5 days 50 mg/kg of Silymarin.

**Group IV- V** test groups were treated in the similar way using ethanolic extract of *Evolvulus alsinoides* (EAEE) 75, 150 mg/kg, respectively like Group-III. Blood samples, liver of the animals were collected and processed in the similar way as mentioned in preventive studies.

**2.6.3. Determination of Sleeping Time**[16]

Pentobarbitone-induced sleeping time was carried out in rats. A 50% v/v $CCl_4$ in olive oil at a dose of 1.5 ml/kg/p.o was used as the toxic substance for inducing liver damage. **Group-I** served as a control and received 1 ml/kg of 1% tween-80 in distilled water p.o. for seven days**. Group-II** served as a toxic control and were given 1% tween-80 for seven days followed by 1.5 ml/kg of $CCl_4$ (50% v/v $CCl_4$ in olive oil) only on the 7$^{th}$ day p.o. The **Group-III** (Standard) animals were administered with 50 mg/kg of Silymarin p.o. for seven days followed by 1.5 ml/kg of $CCl_4$ (50% v/v $CCl_4$ in olive oil) only on the 7$^{th}$ day p.o. **Groups IV,** were treated in the similar way to that of Group III (Standard) using ethanolic extract of *Evolvulus alsinoides* (EAEE 150 mg/kg).

All the various groups of animals were given Pentobarbitone 60 mg/kg i. p. 2 hrs after administration of $CCl_4$. The time between loss of righting reflex and its recovery was recorded (sleeping time).

**2.7. Histopathological examination**

For histopathological studies the liver sections were prepared 3-5 mm thick, stained with alum hematoxylin and eosin and examined microscopically for histopathological changes.

### 2.8. Statistical analysis

Values are expressed in Mean±S.E.M. for six animals in each group and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Dunnett's test. The P<0.05 was considered significant.

## 3. RESULTS

The percentage yield of whole plant **EAEE** was found to be 15.67%. The preliminary phytochemical analysis of EAEE shows the presence of flavonoids, carbohydrates, glycosides, proteins, alkaloids, amino acids, tannins and phenols. Absence of fixed oils and saponins, as summarized in Table.1.

| Tests | Results(Present/Absent) |
|---|---|
| **Alkaloids:** | |
| a.Dragendroff's test | +ve |
| b.Mayer's test | +ve |
| c.Hager's test | +ve |
| d.Wagner's test | +ve |
| **Carbohydrates:** | |
| a.Molish's test | +ve |
| b.Fehling's test | +ve |
| c.Benedict's test | +ve |
| **Flavonoids :** | |
| a.Shinoda test | +ve |
| b.Lead acetate test | +ve |
| **Glycosides:** | |
| a.Borntrager's test | +ve |
| b.Baljet's test | +ve |
| c.Keller-Killani test | +ve |
| d.Picric acid test | +ve |
| **Saponins:** | |
| Foam test | -ve |
| **Tannins:** | |
| a.Ferric chloride test | +ve |
| b.Bromine water test | +ve |

**Table.1:** Preliminary Phytochemical Analysis of ethanolic extract of *Evolvulus alsinoides*

### 3.1. Acute toxicity study

The ethanolic extract of *Evolvulus alsinoides* did not cause any mortality up to a dose of 1500 mg/kg body weight by p.o, and no behavioral, neurological and autonomic profiles and was found to be safe. Hence the maximum and minimum doses i.e 150 mg/kg and 75mg/kg was chosen for the studies.

### 3.2. Hepatoprotective and Anti Hepatotoxic studies in CCl4 induced model:

Based on $LD_{50}$ values the hepatoprotective studies were carried out and the biochemical parameters are given in Table-2 and Fig-1. from the above results. The administration of $CCl_4$ induced by increased the levels of SGPT, SGOT, ALP , CHOL and TBL when compared with the control group. As well as those the animals receive CCl4 cause to decreased the levels of TP and ALB. The test treated groups of EAEE 75 and 150 mg/kg exhibited reduction in the serum levels of SGPT, SGOT, ALP, CHOL and TB (P<0.001). The TP and ALB (P<0.001) levels were also increased and EAEE 150mg/kg dose revealed highly significant when compared to the toxic groups, similar to standard group.

In Antihepatotoxic study Biochemical Parameters given in Table-3 and Fig-2. Of this CCl4 administered group(toxic) showed by increased the levels of SGPT, SGOT, ALP , CHOL and TBL. As well as those the animals receive CCl4 cause to decreased the levels of TP and ALB when compared with the control group. The standard(Silymarin 50 mg/kg), test treated groups of EAEE 75 and 150 mg/kg exhibited significantly reversed the levels of all biochemical parameters when compared to the toxic group. The results of EAEE 150 mg/kg dose revealed highly protective against toxic, almost similar to standard group.

### 3.3. Pentobarbitone-Induced Sleeping Time

The results of this study are given in Table 4. The pentobarbitone at a dose of 60 mg/kg (i.p) caused sedation in rats of control group for a period of 111.7 min, whereas treatment of animals with $CCl_4$ (Toxic group) prolonged the pentobarbital sleeping time to 246.7 min. Prior treatment of animals with EAEE (150 mg/kg), and Silymarin (50 mg/kg) significantly reduction of pentobarbital sleeping time as 181.7 and 138.3.

### 3.4. Histopathological studies

Histological profile of Hepatoprotective studies (fig-3) of liver sections where control animals showed normal hepatocytes, CCL4 induced animals sections showed remarkable changes like centrilobular necrosis, Haemmahroages, Dilation of sinusoidal spaces, bleeding hepatic lobes, Inflammatory cells and macrovesicular fatty changes . Pretreated with EAEE 75 mg/kg, 150 mg/kg groups showed a significant recovery from liver damage. Of these Group V animals treated with EAEE 150 mg/kg exhibited normal architecture, reduced bleeding lobes, reduced necrotic and moderate accumulation of fatty lobules.

Antihepatotoxic studies Histopathological studies liver sections (fig-4), toxic group animals showed remarkable changes like more necrosis, Haemmahroages, Dilation of sinusoidal spaces, bleeding hepatic lobes and macrovesicular fatty changes. Among this EAEE 150 mg/kg group showed a significant recovery from liver damage like lesser necrotic zones, reduced bleeding lobes, lesser dilation of sinusoidal spaces moderate accumulation of fatty lobule s and normal architecture as similar to standard group.

| GROUPS | SGPT (IU/L) | SGOT (IU/L) | ALP (KA/dL) | TB (mg/dL) | TP (gm %) | ALB (gm %) | CHOL (mg/dL) |
|---|---|---|---|---|---|---|---|
| NORMAL CONTROL | 43.25± 5.46 | 48.6±3.61 | 18.66±1.51 | 2.41±1.06 | 8.75±0.29 | 2.58± 0.22 | 79.30±4.87 |
| TOXIC CONTROL ($CCl_4$) | 152.4± 12.31 | 131.7± 12.76 | 78.59±8.15 | 7.25±0.37 | 2.55± 0.14 | 1.11± 0.06 | 148.3±3.19 |
| STANDARD (Silymarin) | 69.75±3.39*** | 75.23±5.09*** | 43.08±2.41*** | 2.75± 0.29*** | 7.09±0.09 *** | 2.45±0.19*** | 99.74±2.39*** |
| EAEE 75 | 107.8±10.56** | 107.9±12.7 | 60.31±4.52* | 5.09±0.05 * | 3.92±0.44 * | 1.70±0.05 * | 132.9±3.07* |
| EAEE 150 | 87.07±5.84*** | 85.20±5.57** | 50.99±3.81** | 3.63±0.26 *** | 5.95±0.40 *** | 2.20±0.11*** | 108.4±3.99*** |

**Table. 2: Hepatoprotective activity of ethanolic extract leaves of *Evolvulus Alsinoides* different biochemical parameters in CCl4 induced liver damage in rats. Graphpad Prism, Version5.0 (Dunnettes method)**

| GROUPS | SGPT (IU/L) | SGOT (IU/L) | ALP (KA/dL) | TB (mg/dL) | TP (gm %) | ALB (gm %) | CHOL (mg/dL) |
|---|---|---|---|---|---|---|---|
| NORMAL CONTROL | 39.42±4.48 | 54.49±3.82 | 18.68±2.0 | 1.02±0.27 | 6.91±0.37 | 4.25±0.2 | 102.6±8.57 |
| TOXIC CONTROL($CCl_4$) | 161±7.38 | 154±9.94 | 72.36±4.74 | 6.59±0.41 | 2.74±0.23 | 1.64±0.14 | 160.3±4.23 |
| STANDARD (Silymarin) | 72.59±4.08*** | 73.92±1.96*** | 41.84±4.26*** | 2.53±0.33*** | 5.60±0.54*** | 2.93±0.30*** | 117.5±4.78*** |
| EAEE 75 | 112±14.75*** | 112±14.75** | 60.47±2.69 | 4.86±0.07** | 3.56±0.39 | 1.99±0.11 | 136.9±3.32* |
| EAEE 150 | 88.11±8.08*** | 78.73±3.95*** | 46.75±1.81*** | 3.352±0.39*** | 5.56±0.34*** | 2.61±0.17** | 128.3±7.16*** |

**Table.3: Hepatotoxic activity of ethanolic extract leaves of *Evolvulus Alsinoides* different biochemical parameters in $CCl_4$ induced liver damage in rats. Graphpad Prism, Version5.0 (Dunnettes method)**

N=6, values expressed as Mean ± SEM. Significant*(P <0.05), ** (P<0.01), *** (P<0.001) compared with standard and toxic group.

| GROUPS | SLEEPING TIME(Min) | % REDUCTION IN SLEEPING TIME |
|---|---|---|
| CONTROL | 111.7±7.27 | -- |
| TOXIC | 246.7 ± 10.14 | -- |
| STANDARD(50 mg/ kg) | 138.3 ± 7.27*** | 23.81 |
| EAEE-150 | 181.7 ± 10.14** | 62.66 |

**Table.4: Effect of EAEE on pentobarbitone sodium induced sleeping time in CCl4 intoxicated rats.**

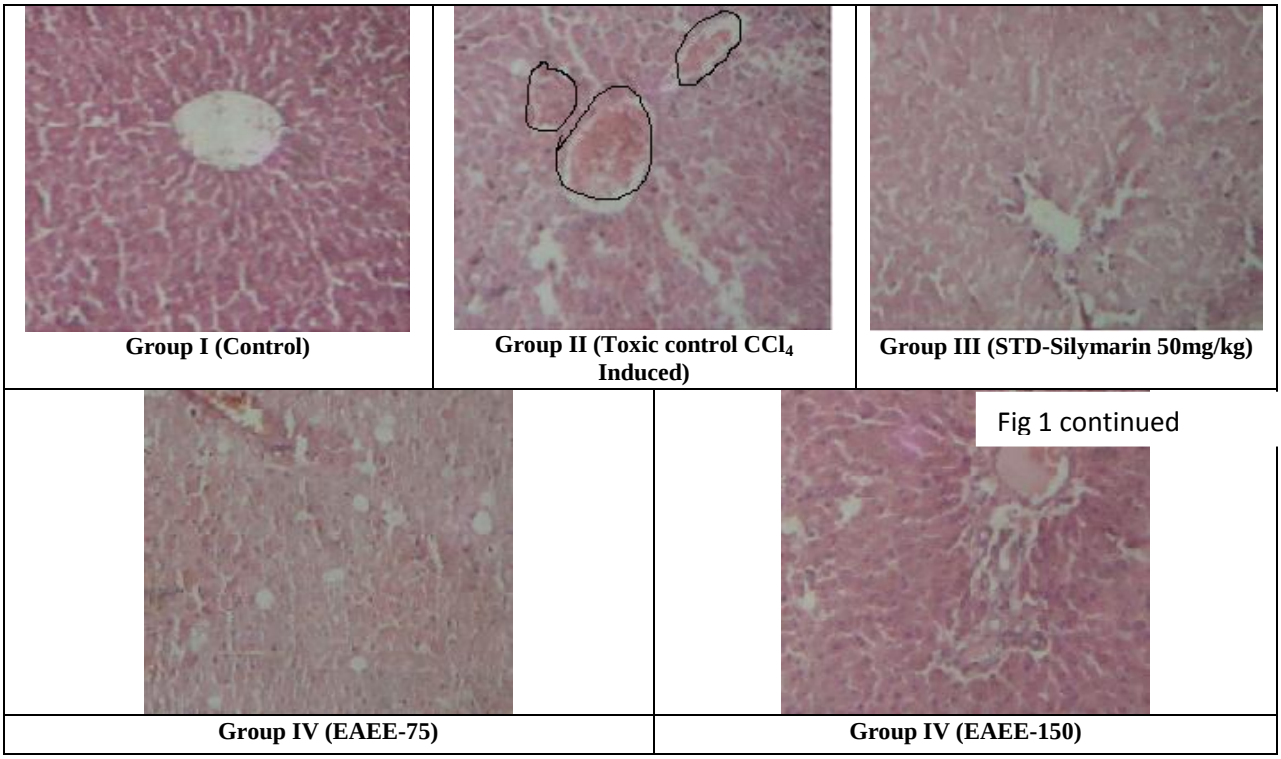


**Fig.1: Effect of EAEE pre-treatment on histopathalogical changes in $CCl_4$ induced liver damage in rats.**

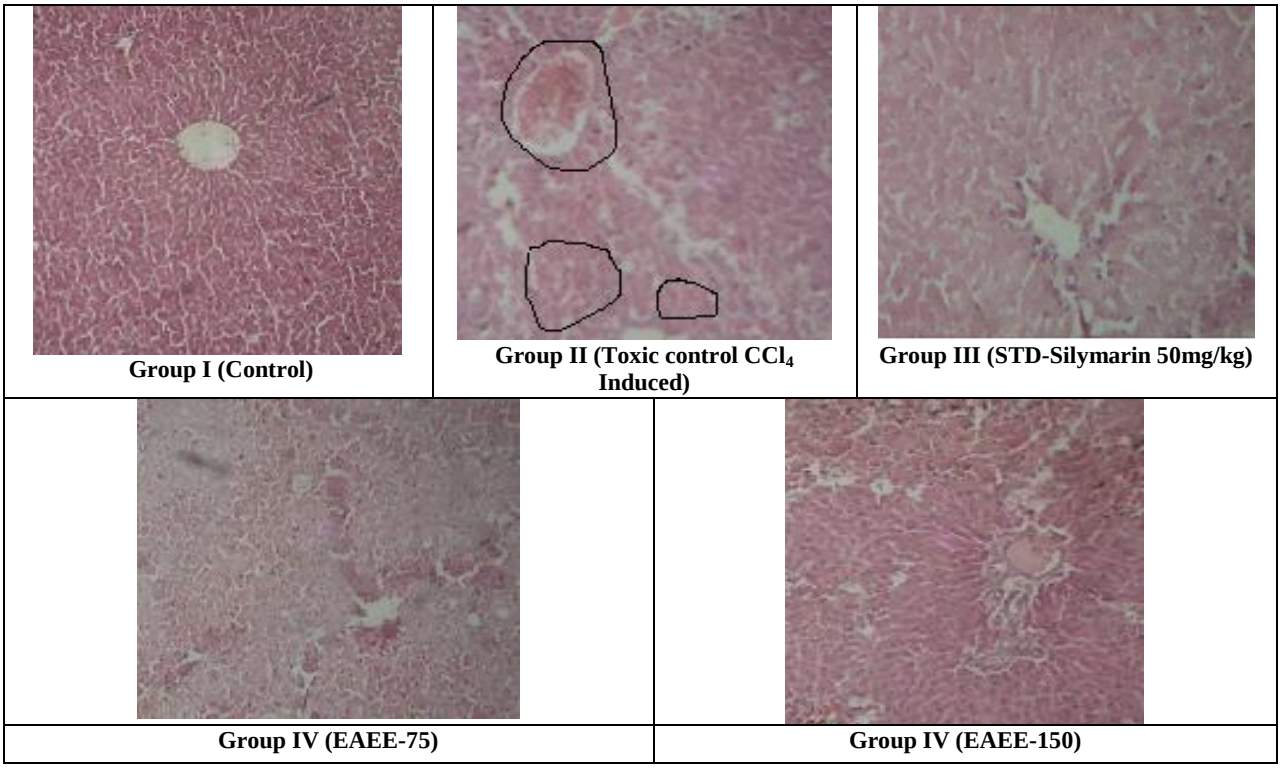


**Fig.2: Effect of EAEE post-treatment on histopathalogical changes in CCl4 induced liver damage in rats.**

## 4. DISCUSSION

*E. alsinoides* L. is traditionally used as medicine in East Asia, and also is in Ayurveda as a brain tonic in the treatment of neurodegenerative diseases, asthma, malarial fever, nervous debility, growth hair, loss of memory, syphilis and amnesia[17]. In Southern Western Ghats of India, whole plant of *E. alsinoides* is used for the treatment of venereal diseases[18], spermopiotic[19]. Plant contains alkaloids: betaine, shankhapushpine and evolvine. The fresh plant contains volatile oil, yellow

neutral fat. An unidentified compound has been isolated.[17] Scopoletin, scopolin, umbelliferone, 2-methyl-1,2,3,4-butanetetrol, ferulic acid esters with alcohols $C_{14}$-$C_{17}$ and palmitic, stearic, oleic, 8-methyldecanoic and heptadecanoic acids reported.[20,21] 2,3,4-tri hydroxy-3-methylbutyl 3-[3-hydroxy-4-(2,3,4-trihydroxy-2-methylbutoxy)-phenyl]-2propenoate, kaempferol-3-O-β-glucopyranoside and quecetine-3-O-β-glucopyranoside were reported from *n*-BuOH soluble fraction from the ethanol extract of *E. Alsinoides.*[22]

In view of above claims and facts, the present work was undertaken as a research study to prove the usage of the plants in the treatment of liver damage scientifically. In the present investigation, ethanolic extracts of *E. Alsinoides* were screened for hepatoprotective and antihepatotoxic activity in rodents. The physical status and percentage yield of ethanolic extract of whole plant of *E. alsinoides* were recorded for future reference.

In acute toxicity study, no mortality occurred within 48 hrs upto a dose level of 1500 mg/kg b.w.p.o. with ethanolic extracts of *E. alsinoides* (EAEE). The $LD_{50}$ was therefore, greater than 1500 mg/kg in mice. Hence, the pharmacological studies of these extracts were carried out with 2 doses i.e., 75 and 150 mg/kg b.w.p.o.

The hepatoprotective model and antihepatotoxic model viz. $CCl_4$, were used for liver damage induction. The results of the studies demonstrate that the various biochemical changes were produced in serum as well as liver and histological changes of liver by $CCl_4$ toxicity were prevented or reversed by administration of EAEE. The $CCl_4$ administration to rats leads to marked elevation in the levels of serum SGOT, SGPT and ALP. This might be due to the release of these enzymes from the cytoplasm of hepatic cells, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage[23], resulting from the $CCl_4$-induced lipid peroxidation[24]. Treatment with EAEE at 75 and 150 mg/kg b.w.p.o. significantly reduced the level of these marker enzymes in $CCl_4$ treated rats in hepatoprotective study. The decrease in the levels of these enzymes may be a consequence for the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $CCl_4$[25]**.** In $CCl_4$ induced hepatotoxicity, elevated serum TB level is due to defective excretion of bile by the liver indicates the loss of integrity of liver and necrosis. This leads to increase in the binding, conjugating and excretory capacity of hepatocytes, which is proportional to the erythrocyte degeneration rate[5]**.** At both the test doses EAEE showed a significant depletion in the serum bilirubin level suggesting the possibility of the extracts ability to stabilize biliary dysfunction of rat liver during injury with $CCl_4$ in prophylactic and curative studies. In $CCl_4$ hepatotoxicity, a depression in total protein occurs due to the disruption and dissociation of polyribosome on endoplasmic reticulum leading to defective protein biosynthesis[26,27]**.** EAEE at 75 and 150 mg/kg doses, increased the serum TP and ALB levels with varying degree of significance. This may be due to the promotion of the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis. The overall observation made on the biochemical parameters revealed that the extract have better protection activity with the dose i.e. 150 mg/kg b.w.p.o., similar to that of the reference drug, Silymarin (50 mg/kg).

The histopathological profile of the liver of $CCl_4$ administered rats revealed drastic alterations in histoarchitecture showing centrilobular necrosis, fatty changes, broad infiltration of lymphocytes Dilation of sinusoidal spaces and bleeding hepatic lobes. In hepatoprotective and antihepatotoxic studies; EAEE at both test doses showed definite signs of protection and recovery against $CCl_4$ injury respectively. Among this EAEE150 mg/kg exhibited a remarkable recovery towards normalization of histological architecture of livers of the rats, which was almost similar to that of Silymarin (50mg/kg).

EAEE (150 mg/kg) was substantiated by pentobarbitone (60 mg/kg) sleeping time experiment in rat. Pentobarbitone is metabolized by hepatic microsomal drug metabolizing enzyme (MDME) to inactive metabolites and any drug with an inhibitory effect on MDME is expected to prolong pentobarbitone-induced sleep time (Fujimoto *et al.,* 1960). The damage conferred by $CCl_4$ on hepatocytes as well as on the hepatic MDME causes a loss of drug metabolizing capacity of the liver, resulting in prolongation of pentobarbitone induced sleep time[28]. The EAEE, Standard drugs reduce sleeping time because of recovery of hepatic enzymes(MDME). The EAEE 150 mg/kg, reference drug Silymarin (50 mg/kg) was showing similar responses .

It is evident from the results that the biochemical, sleeping time and histopathological observations of the studies were complementing each other. This confirms that ethanolic extract of *E. alsinoides* (EAEE) 150 mg/kg, have hepatoprotective and antihepatotoxic effect in $CCl_4$- induced toxicity in rats by their ability to stabilize cell membranes, scavenge free radicals and antioxidant properties.

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