

Hepatoprotective activity of Alcoholic leaf extract of *Alianthus exelssa* root against Carbon tetrachloride toxicity induced in albino rat *Rattus rattus* (Wistar)

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Abstract: The ethanolic extracts of roots of *Alianthus exelssa* belonging to the *Simaroubaceae* family were studied for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride (CCl₄). It was found that the ethanolic root extract of *Alianthus exelssa* at a dose of 100 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of Alkaline Phosphatase (ALP), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxalate Transaminase (SGOT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and cholesterol (CHL) to a significant extent. The highest activity was observed for ethanolic root extract of *Alianthus exelssa* at a dose of 200 mg/kg body weight (b.wt.). The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme, reflecting the liver injury caused by CCl₄ and blood samples from the animals treated with the ethanolic root extracts of *Alianthus exelssa* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells. Thus the extracts of *Alianthus exelssa* could afford significant dose-dependent protection against CCl₄ induced hepatocellular injury.

Key words: *Alianthus exelssa*, Hepatoprotective activity, Carbon tetrachloride.

1. Introduction:

The liver is one of the few organs of highly specialized function whose cells can undergo an astonishing degree of regeneration. Modern allopathic treatment does not hold promise to cure liver disease perfectly. A number of medicinal plants are used in traditional system of medicine for the management of liver disorders¹. *Alianthus exelssa* is one such medicinal plant used in the treatment of liver disorders in folk medicine. During the past decade, the indigenous or traditional system has gained importance in the field of

medicine. In most of the developing countries, a large number of population depend on the traditional practitioners, who are dependent on medicinal plants to meet their primary health care needs. Although, modern medicines are available, herbal medicine retained their image for historical and cultural reasons. Since the usage of these herbal medicines has increased, issues and moto regarding their quality, safety and efficacy in industrialized and developing countries have cropped up². Increasing interest has forced researcher to screen scientifically various traditional claims. There is need of screening the

traditional claims because this is scientific era and everyone wants the scientific support before using the traditional drugs. So presently both common user and health care professionals seek updated, alternative information towards safety and efficacy of any recommended medicinal plants as drug prior to its use. The aim of the present context is to evaluate the hepatoprotective potential of the ethanolic extract of *Alianthus excelsa* root in a conventional animal model of hepatopatoxicity.

Ailanthus excelsa is a deciduous tree from the Simaroubaceae family and widely distributed in Asia and northern Australia. Its native origin is China and is known as the "tree of heaven"³. In traditional medicine *A. excelsa* is used to cure wounds and skin eruption and is used in the indigenous system of medicine in febrifuge, bronchitis, asthma and in conditions of diarrhea and dysentery in Indian system of medicine⁴ *Ailanthus excelsa* Roxb. is used in the treatment of asthma, bronchitis, cough, cold^{5,6}. Tribals in Nilgris region traditionally used it in antifertility, fever. Studies on stem bark extracts have shown reduced labour pain, febrifuge⁷, antispasmodic⁸, anticancer, antimicrobial, antiamebic, antiprotozoal activities^{9,10} and antiasthmatic activity¹¹.

2.0 MATERIALS AND METHODS

2.1 Plant material:

The root part of plant *Alinthus excelsa* plant was collected from young matured plant from the rural belt of Yavatmal District Maharashtra during the month of Jan-Feb and identified by botanist of Department of Botany, Government Institute of Science and Humanities, Amravati by comparing with the voucher specimen present in the herbarium. After authentication fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder was used for further studies.

2.2 Animals:

Healthy albino male rats of Wistar strain weighing between 150-200 g were selected for the investigation (Brought from Sudhakarao Institution of Pharmacy, Pusad, Maharashtra, India). The animals were kept under maintained uniform laboratory conditions (12 ± 1 hr, day and night schedule; temperature maintained between 11-20⁰ ± 2⁰ C; housed in large spacious hygienic cages) before a week of the experiment for acclimatization. The protocol of present study was approved by Institutional animal ethical committee constituted as per CPCSEA guidelines (1060/ac/07/CPCSEA/Dec2009).

2.3 Chemicals:

Chemicals used in the study were of analytical grade and were procured from Merck Specialties private limited, Mumbai, India. All biochemical assay kits were purchased from Ecoline: Merck specialties private limited, Mumbai, India.

2.4 Extraction of plant material and preparation of test dose:

About 200 gm of coarse dried powder of root of the *Alianthus excelsa* was taken in the soxhlet apparatus and extracted¹² using the selected solvent Ethanol. The extraction was carried out for 18 to 20 hours. The extract was collected by evaporating the solvents by slow heat treatment. Total 1.4kg of pulverized root bark was subjected under solvent extraction to produce the required amount of test extract. Calculated amount of dried ethanolic extract was suspended in 0.5% w/v of sodium- CMC in normal saline solution to get the test doses (200mg/kg and 400mg/kg per ml.). The dose limits were selected on the basis of previously performed oral acute toxicity studies in rat, in accordance with the OECD guidelines¹³.

2.5 Phytochemical analysis:

The preliminary phytochemical analysis of ethanolic extract of root of *A.excelsa results* showed the presence of alkaloids, tannins, flavonoids, terpenoids, quassinoids, carbohydrates and sterols.

2.6 Acute toxicity studies:

In the acute toxicity test carried out in rat we took six doses and 10 rats were administered each dose of both aqueous and ethanolic extract i.e. 500, 1000, 1500, 2000, 2500, 3000 mg/kg body weight. All groups of test drug showed neither any toxic effect nor any lethal effect in the dose range of 500 to 3000 mg/kg body weight. However 3000 mg/kg of ethanolic extract showed altered behavior of some mice. So a minimum and maximum dose of 100mg/kg and 200 mg/kg of body weight was taken for ethanolic extract for further screenings.

2.7 Hepatoprotective activity study:

30 rats were divided randomly into 5 groups, each group comprising 6 animals.

Group I(Normal Control) received oral dose of 0.5% Sodium CMC (1 ml each) for 7 days. **Group II**(Toxic Control) received single dose of CCl₄ (CCl₄ + Olive oil in 1:1 ratio; 2ml/kg of body wt; i.p.) on day 1 and day 7 of the experiment^{14,15}.

Group III, IV and V received standard drug ,, Sylimarin (25 ml/kg; p.o.) Sylibon micro lab., Bangalore, India), ethanolic extract 200 mg/kg of body wt. and 100mg/kg of body wt. once in a day for 7 days

respectively, along with the i.p dose of CCl₄ on day 1 and day 7 as mentioned above. The treatment duration was of 7 days. On the 8th day of the study the animals were sacrificed under anesthesia and blood samples were collected from each animal to produce the serum for biochemical assay. The biochemical investigations were performed by using a Biochemical semi auto analyzer (EBRA-Chem-5 Plus. V2., West- Germany). The biochemical parameters considered were: Serum SGOT (Glutamate oxalate transaminase), SGPT

(Glutamate pyruvate transaminase), ALP (alkaline phosphatase), Serum albumin and TPTN (total protein)^{16,17,18}. Results of all the estimations done were indicated in terms of Mean \pm SEM. Statistical significance of data were assessed by analysis of variance (One Way- ANOVA)¹⁹, The significance was set at the level of $p < 0.05$. The toxic control group was compared with the normal control group and all other treatment groups were compared with the toxic control group.

Table I. Chemical constituents of leaf and root extracts of *A. excelsa*.

Chemical Constituents	A. excelsa B. Root extract
Carbohydrates and/or glycosides	+
Condensed tannins	+
Hydrolysable tannins	-
Alkaloids and/or nitrogenous bases	+
Flavonoids	+
Sterols and/or	+
triterpenes	+
Saponins	-
Coumarins	+

(+) presence of the constituent (-) absence of the constituents.

Table II: Effect of ethanolic extract of root bark of *Ailanthus excelsa* Roxb. on serum enzymatic levels in rat

Treatment Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (g/dL)	TPTN (g/dL)	CHL (IU/L)
Normal control	155.6 \pm 3.82	66.56 \pm 11.05	198.25 \pm 0.52	0.75 \pm 0.14	14.17 \pm	108.66 \pm 1.11
CCl ₄ Control	316.40 \pm 12.50	179.22 \pm 4.26	495.50 \pm 9.27	1.22 \pm 0.17	7.81 \pm 0.05	229.67 \pm 2.45
Silymarin treated (25mg/kg/p.o)	172.10 \pm 8.33 ^{***}	73.47 \pm 2.39 ^{***}	220.08 \pm 6.36 ^{***}	0.75 \pm 0.02 ^{***}	10.65 \pm 0.06 ^{***}	174.30 \pm 1.17 ^{***}
Ethanolic extract treated (100mg/kg/p.o)	213.50 \pm 1.62 [*]	103.45 \pm 2.45 [*]	276.25 \pm 2.54 [*]	0.84 \pm 0.22 [*]	6.07 \pm 0.012 [*]	141.65 \pm 1.94
Ethanolic extract treated (200mg/kg/p.o)	198.02 \pm 5.78 ^{**}	87.05 \pm 2.61 ^{***}	210.30 \pm 7.01 ^{***}	0.75 \pm 0.22 ^{***}	7.21 \pm 0.081 ^{***}	167.29 \pm 0.73 ^{**}

N=6. The results were expressed as mean \pm S.E.M. $p < 0.001$ was considered as significant. The significance between the groups was verified by one way analysis of variance (ANOVA)

Markers from Different Groups Compared with Control Group

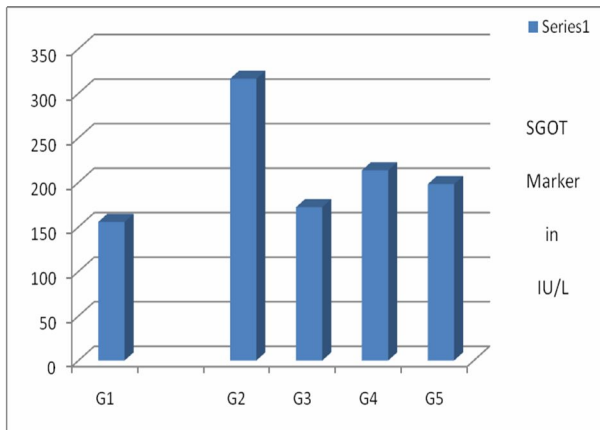


Figure I (SGOT Marker)

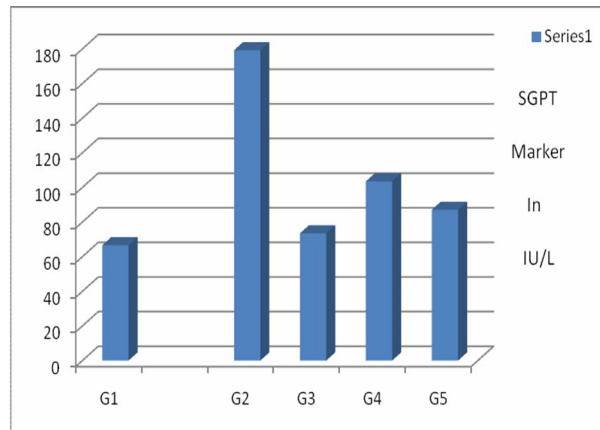


Figure II (SGPT Marker)

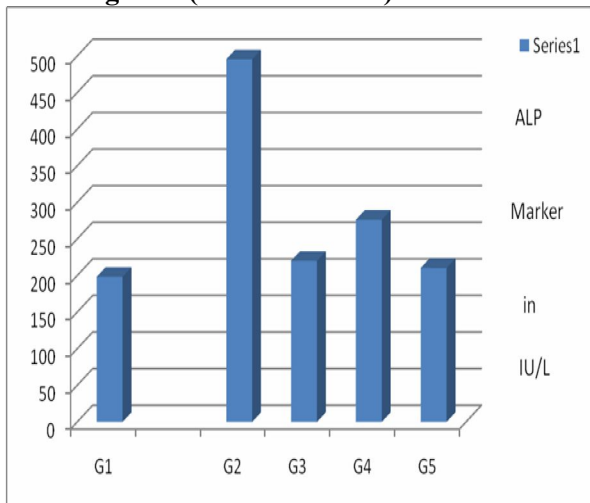


Figure III (ALP Marker)

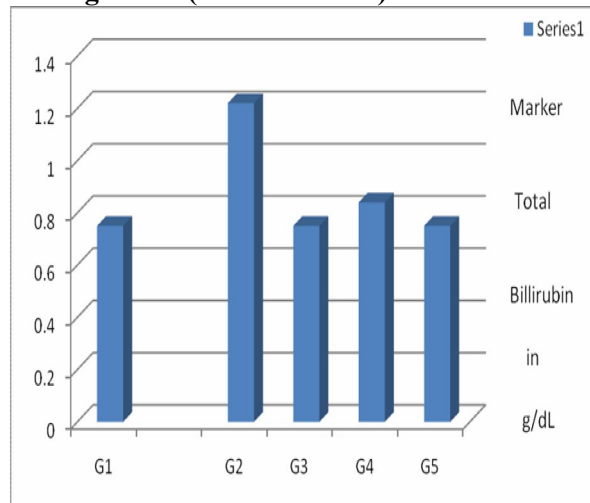


Figure IV (TB Marker)

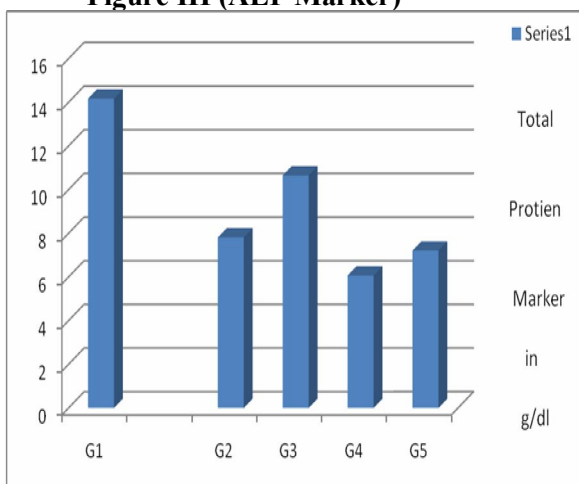
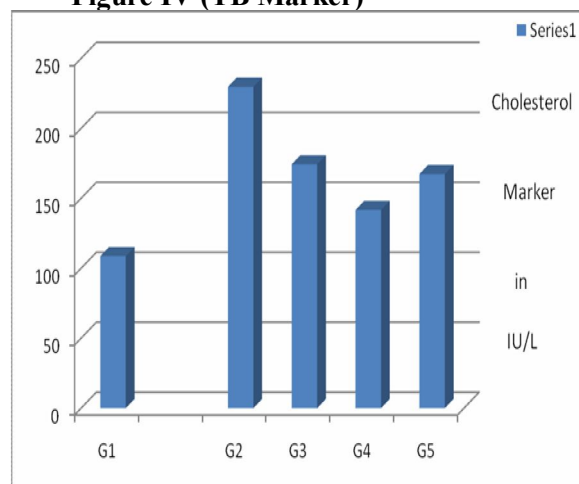
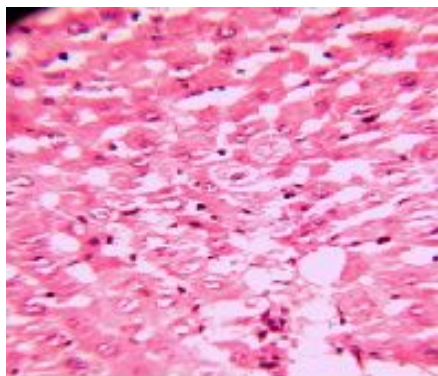
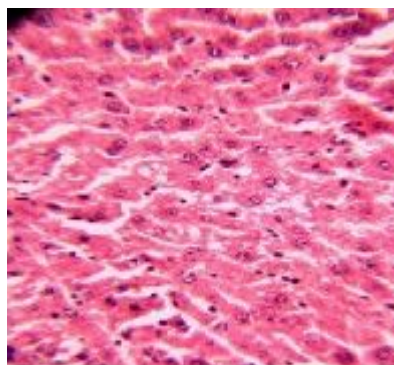
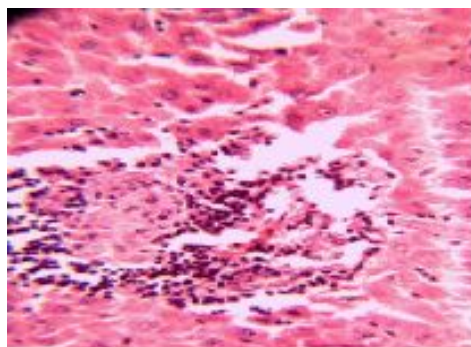
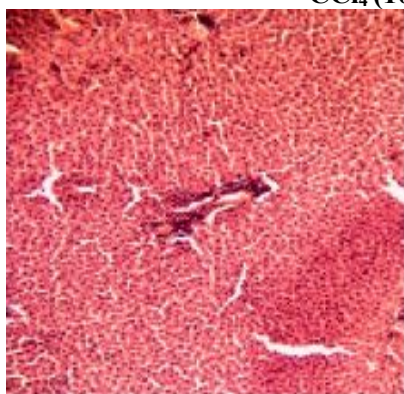
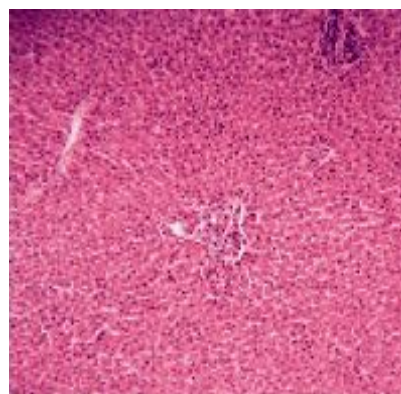


Figure IV (TPTN Marker)



FigureV (CHL Marker)

Figures showing sections of different group:**100mg(100x)****200mg(100x)****CCl₄ (100x)****Silymarin(100x)****Control (100x)****3. Results:**

Preliminary phytochemical studies revealed the presence of alkaloids, steroids, saponins, triterpenes, flavonoids and polyphenolic compounds. For the acute oral toxicity studies, the extract treated animals were observed for mortality up to 48 h. Based on the results it was observed that the extract did not produce any mortality up to 2000 mg/kg body weight.

3.1 Effect on serum enzymatic activity

An alternation in the activities of serum enzymes (SGOT, SGPT, ALP and TB) content in the serum of CCl₄ induced liver damage in rats was seen evidenced from Table 1. The level of serum marker enzymes SGOT, SGPT, ALP and TB were found to be

significantly increased in CCl₄ induced liver damaged rats when compared with the normal group ($p < 0.001$), whereas treatment with ethanolic extract of stem bark of *A. excelsa* at the dose of 100 and 200mg/kg/p.o. showed decrease in the elevated serum enzyme levels in CCl₄ induced liver damage in rats compared to that of control groups ($p < 0.001$). Silymarin (25mg/kg/ p.o.) also significantly decreased the levels of serum enzymes and bilirubin content in CCl₄ treated groups as compared with the respective control.

3.2 Histopathological Study:

Histopathological examination of liver sections of normal control group (Fig 1) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In the liver section

of rats intoxicated with CCl₄ (Fig 2), there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis extending to midzone and sinusoidal haemorrhages and dilation. The liver section of the rats treated with 100 and 200 mg/kg/p.o.(group III &IV) and Silymarin 25mg/k/p.o.(group V) showed less vacuole formation, reduced sinusoidal dilation and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. The centrilobular necrosis was also less (Fig 3, 4 & 5).

4. Discussion:

CCl₄ is one of the most commonly used hepatotoxins in experimental studies of liver diseases²⁰. CCl₄ is potent hepatotoxin, and single exposure to it can rapidly lead to an increase in the level of several enzymes, severe centrilobular necrosis and steatosis (21). The hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloro methyl radical²², a free radical which binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum²³. Hepatocellular necrosis leads to very high serum levels of GOT and GPT which are released from liver into the blood. Among the two, GPT is a better index of liver injury, as liver GPT activity represents 90 % of total enzyme present in the body²⁴. ALP activity on the other hand is related to hepatocyte function. An increase in its activity is due to increased synthesis in presence of increased biliary pressure²⁵.

In this study, we used CCl₄ induced liver toxicity that is frequently used as model to study hepatoprotective activity of drugs²⁶. Silymarin was used as standard drug, Silymarin, a standardized extract of *Silybum marianum* (Compositae) is also a potent hepatoprotective agent. It reverses hepatotoxin-induced alterations of biochemical parameters and has so far been the most thoroughly investigated of all the plant substances in preventing liver damage induced by carbon tetra chloride, D-galN and paracetamol in rat models²⁷.

The ability of hepatoprotective plant drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by carbon tetrachloride administration was observed by recording SGOT, SGPT, SALP and SBLN levels in different groups²⁸. Although both the doses of ethanolic extracts (100mg and 200 mg/Kg b.w.) offer hepatoprotective activity, the higher dose (200mg/Kg b.w.) is more effective (Table II). Reduction in the levels of GOT, GPT, total protein and cholesterol towards their normal values is an indication of stabilization of plasma membranes as well as repair of hepatic tissue damage.

The Histological studies also support the efficacy of drug as a hepatoprotective agent. Simultaneous treatment of ethanolic extract with CCl₄ produces lesser degree of damage to liver cells as compared to the animals treated with CCl₄ alone. The section of the liver treated with extract (200mg/Kg b.w.) and CCl₄ reveal better hepatoprotective activity almost similar to the standard (silymarin) group. Negligible damage to a few hepatocytes present in the close vicinity of the intra lobular vein is observed. Hepatocytes show normal appearance with the presence of vacuoles in the cytoplasm. Thus data clearly shows that the hepatoprotective activity of *Alianthus exelsa* and justifies the use of this plant for the treatment of jaundice.

Preliminary phytochemical screening revealed the presence of glycosides, Condensed tannins, Flavonoids, Sterols and triterpenes. Further isolation of the active principles responsible for the hepatoprotective activity of *Alianthus exelsa* plant is currently in progress within our laboratory.

5. Conclusion

In the present pharmacological evaluation the root extracts (ethanolic) of *Alianthus exelsa* plant was extensively investigated for its Hepatoprotective potential against substance (CCl₄) induced hepatopathy. At the end of our study, a strong conclusion can be drawn that, the ethanolic extract of *Alianthus exelsa* possess Hepatoprotective activities more or less depending on the dose levels. The ethanolic extract of the plant at a dose level of 250mg/kg exhibited competent, potent and comparable results whereas ethanolic extract at a dose level of 150mg/kg, was observed to have moderate level of efficacy, promoting *Alianthus exelsa* plant as a promising Hepatoprotective plant species, seeking vast multidimensional future research work up to the molecular level to establish new up-to-date scientific data about this plant species and to elucidate its exact mechanism of protective effect. Future studies may be aimed at hepatoprotective study in other chronic models of Hepatopathies, antioxidant and free radical scavenging potentials, toxicological studies and other pharmacological activities as well.

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