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Original Research Article (Experimental)

Hepatoprotective effect of *Lobelia alsinoides* Lam. in Wistar rats

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A B S T R A C T

Background: Traditional healing practitioners of South India use fine paste (an Ayurvedic dosage form known as '*kalka*') of *Lobelia alsinoides* Lam., an ethno medicinal plant for curing hepatic diseases.
Objective: To evaluate *in-vivo* hepatoprotective effect of a candidate formulation *viz. kalka* containing whole plant (*L. alsinoides* Lam.) in rat model of Carbon-tetrachloride (CCl_4) induced hepatotoxicity.
Materials & methods: Hepatotoxicity was induced in Wistar albino rats by oral administration of 1.25 ml/kg CCl_4 once every day for 7 consecutive days. A candidate *kalka* formulation (fine paste) was prepared and administered to rats at different dose rates of 0.54 g/kg, 1.08 g/kg and 2.16 g/kg daily. At the end of the study-period, the serum levels of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, albumin and total cholesterol were monitored. Further, the hepatic pathology was evaluated for assessing the extent of hepatotoxicity in the control and hepatoprotective effect in treatment groups. Meanwhile *in-vitro* antioxidant activity of *kalka* was evaluated by hydroxy radical, nitric oxide and DPPH (2, 2 diphenyl-1-picrylhydrazil) radical scavenging assays. Further, a 'limit test' was done in accordance with OECD Guidelines 425 (acute toxicity).
Results: The animals treated with the fine paste of *L. alsinoides* did not show an elevation in the biochemical values compared to CCl_4 treated rats and during histomorphologic evaluation, hepatoprotective effect was evident with scattered mitotic figures in the parenchyma. Acute toxicity evaluation indicated that doses up to 2500 mg/kg are not toxic to rats. It has a good anti-oxidant activity also.
Conclusions: From the study, it was obvious that *L. alsinoides* had significant hepatoprotective effect in CCl_4 induced liver toxicity in rats. This ethno medicinal plant is certainly a promising hepatoprotective drug in liver disorders.
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1. Introduction

Liver diseases have become a global concern worldwide and deaths caused by liver diseases are rising each year at an alarming rate. Many medical management strategies have been proposed to address liver diseases but the search for novel methods and formulations for treating hepatic disorders continues [1]. In this regard, medicinal preparations made from plants that are used for thousands of years by different ethnic communities all over the world [2,3].

Indeed Ayurveda, an Indian system of medical practice shares an exhaustive collection of documented literature over thousands of years on the use of these medicinal plants and various formulations for curing liver diseases [4,5]. The usage of range of plants their derivatives and the suggested formulations as medical preparations vary extensively and be influenced by numerous aspects including the style/system of the practice and ethnic traditions [6]. Traditional Ayurvedic practitioners in Travancore part of Kerala, India use *kalka* (an Ayurvedic dosage form of fine paste) formulation of a plant, locally named as *Cheriya manganari* (*Lobelia alsinoides* Lam.,

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Lobeliaceae), for relieving jaundice. This ethno medicinal plant having cosmopolitan distribution is a perennial herb, with milky white latex [7] seen in marshy areas. It grows abundantly in the western ghats in India [8]. It has been classified as a wild food resource in Thailand [9].

According to Ayurveda, *pitta dosha* is the energy principle in the body which is responsible for all types of biotransformation and controlling digestion, metabolism and energy production. Disorders of *pitta dosha* mainly involve diseases relating to liver. The *Hortus Malabaricus* authored by Hendrik Van Rheede is a compilation of folklore practices, traditional ethno medicinal knowledge and natural plant wealth of Kerala published in twelve volumes during 1678–1693 [10]. This book contains an elementary description about *L. alsinoides* Lam. and its use for treatment of *pitta* disorders. The plant is used therapeutically in *pitta* disorders along with other plants *Kakamachi* (*Solanum nigrum* L.) and *Mandukaparni* (*Centella asiatica* L.) [7]. The medicinal use of this herb is not described in any other classical text books of Ayurvedic literature. Besides, the data on the use of *L. alsinoides* Lam. for preparing modern Ayurvedic formulates are not available in contemporary literature.

Against this background, in this study, *in vivo* hepatoprotective effect of *L. alsinoides* Lam. has been evaluated in an albino rat model of Carbon-tetra chloride induced hepatotoxicity. Acute toxicity, *in-vitro* antioxidant activity and phytochemical properties were also assessed as a part of the study.

2. Materials and methods

2.1. Plant material collection & identification

Mature plant of *L. alsinoides* Lam (Fig. 1A) was collected from its natural habitat in Chirayinkeezhu (Kerala state, India, 8°66′49.9″N 76°78′55.9″E) and the identification was authenticated by the Department of Botany, University of Kerala, Thiruvananthapuram, India. The voucher specimen (No. 1022/DG/AVC) was kept in medicinal plant garden of the Government Ayurveda College, Thiruvananthapuram, India (Fig. 1B).

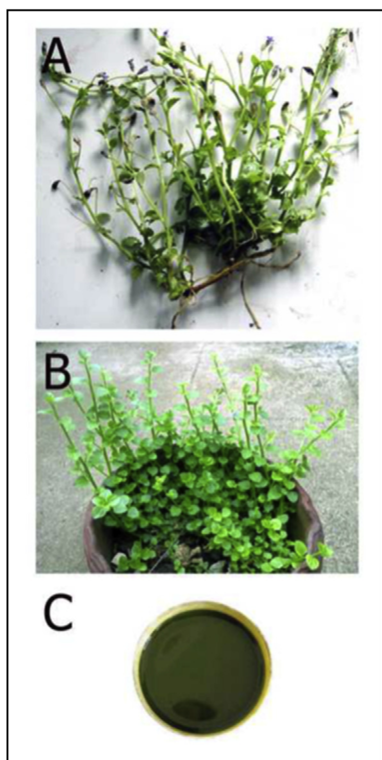

Fig. 1. *Lobelia alsinoides* Lam.; (A) Whole plant (B) Voucher specimen (C) and *Kalka* preparation (fine paste).

2.2. Method of preparation of fine paste (kalka)

Fresh whole plant of *L. alsinoides* Lam. was collected, cleaned, cut into small pieces and 6 g was taken daily and made into fine paste by grinding at a rate of 100 rotations per minute using an electronic stone-mortar and pestle for 1 h (particle size: 8–15 μm, Fig. 1C) to yield 4.5 g *kalka* (fine paste). Everyday a new plant was taken and the *kalka* preparation was done as per standard procedure mentioned in Ayurvedic Pharmacopoeia of India [11].

2.3. Animals

Thirty six healthy adult Wistar albino rats of either sex with body weight between 200 and 250 g were procured from animal house of the Government Ayurveda College, Thiruvananthapuram, India. The animals were housed in polypropylene cages at controlled temperature (22 ± 2 °C) with a 12 h light/dark cycle with standard laboratory pelleted rat feed and water *ad libitum*. The experimental protocol had the approval of the Institutional Animal Ethics Committee of Govt. Ayurveda College, Thiruvananthapuram (Order no: IAEC No.13/IAEC/AVC/2012).

2.4. Acute toxicity study

Acute toxicity study of *kalka* preparation of *L. alsinoides* Lam. was performed as per fixed single dose procedure (limit test) adopted by the Organization for Economic corporation and Development-OECD guidelines 425 [12]. Five healthy nulliparous female Wistar albino rats of 10–12 weeks old, weighing 120–160 g were selected for the study and were deprived of feed overnight and 3 h after the administration of *kalka*. The test dose of 2500 mg/kg of *kalka* was administered orally and were observed individually for mortality (twice daily) and toxic symptoms at 10 min, 30 min, 1 h, 2 h, 4 h and 6 h and once daily thereafter for 14 days. At the end, animals were sacrificed, gross pathological changes were observed and histomorphological examination of liver, kidney and spleen were carried out.

2.5. CCl_4 induced hepatotoxicity

The acclimatized animals were divided into six groups (six animals per group). Animals of Group I was fed normal diet and water *ad libitum* and did not receive any drug treatment. All animals of Group II, Group III, Group IV, Group V and Group VI received 1:1 mixture of CCl_4 (Merck, Germany) in olive oil as single dose of 1.25 ml/kg/day orally for 7 consecutive days as reported previously for inducing hepatotoxicity [13]. In addition, Group II rats received distilled water 1 ml/kg (oral) and Group III rats were treated with Silymarin, the known hepatoprotective compound at a dose of 100 mg/kg (Micro Labs Ltd, Banglore) everyday (oral) [14] for preventing hepatotoxic effect of CCl_4. All animals of Group IV, Group V and Group VI were administered recently prepared fine paste of *L. alsinoides* (*kalka*) (less than 1 h) for seven consecutive days at dose rates of 0.54 g/kg, 1.08 g/kg and 2.16 g/kg respectively as oral bolus mixed in 2 mL of distilled water. Dose was fixed as per *Sarngadhara Samhita*, an Ayurvedic text book on pharmaceutics [15]. According to this, the dose of *kalka* preparations is 12 g/day for man. Based on human to rat dose conversion table [16] the expected therapeutic dosage (per os) for rats is 1.08 g/kg and is given

to Group V. Its half dose (0.54 g/kg) and double dose (2.16 g/kg) were given to animals in group IV and Group VI respectively. Hepatoprotective effect of *kalka* was assessed by serum biochemical analysis and histomorphological studies.

2.6. Serum biochemical analysis

On 8th day, blood was collected from the ocular sinus and the following serum enzymes were analyzed in serum using commercial assay kits obtained from Erba diagnostics, Germany: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, serum albumin, total protein and total cholesterol. Then the rats were sacrificed by cervical dislocation, abdomen was dissected and gross morphological lesions in liver were noted. Liver tissue samples were excised and immersed immediately in neutral buffered formalin.

2.7. Histomorphology

Liver tissue samples collected at necropsy were processed for histopathology using an automated tissue processor (Leica, Germany) and paraffinized tissue blocks were prepared. Thin paraffin sections were cut at 4μm thickness using a semi-automated microtome (Leica, Germany). Tissue sections were then stained with Hematoxylin and eosin (H&E) and were examined under Olympus microscope BX57 and images were captured at 10× and 40× magnifications using DP71 digital camera system.

Histopathology was evaluated both qualitatively and quantitatively under the supervision of a qualified veterinary pathologist. The extents of vacuolar degeneration and necrosis were assessed by semi quantitative scoring criteria (Grade 0; for absence or less than 5% area affected, Grade 1; mild/minimal or less than 25% area affected, Grade 2; moderate or about 50% area affected and Grade 3; severe or more than 50% area affected). In addition, the number of mitotic figures was counted in the entire histology section and was expressed as number per unit area.

2.8. In-vitro anti-oxidant activity

2.8.1. Hydroxyl radical scavenging activity

Different concentration of fine paste of *L. alsinoides* 125–2000 μg/mL from a stock concentration of 10 mg/mL mixed with 500 μL reaction mixture was made up to a final volume of 1 mL. After incubation for 1 h at 37 °C, add 1 ml of 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90 °C for 15 min to develop the color. After cooling the absorbance was measured at 532 nm against an appropriate blank solution and Gallic acid (125–2000 μg/mL concentrations from a stock concentration of 10 mg/mL) was taken as the standard. IC_{50} value was evaluated using IC_{50} PLUS V1.0 Software [17].

2.8.2. Estimation of nitric oxide radical scavenging activity

Sodium nitroprusside (5 mmol L^{-1}) was mixed with different concentration of the fine paste of *L. alsinoides* 125–2000 μg/mL from a stock concentration of 10 mg/mL and incubated at 25 °C for 30 min in phosphate mixture. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent. Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-(1naphthyl) ethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard, Gallic acid (125–2000 μg/mL concentrations from a stock concentration of 10 mg/mL) [18].

2.8.3. Estimation of DPPH radical scavenging assay

A methanol solution of (1.48 mL) DPPH (0.04 g/L) was added to the different concentrations (12.5 μg/mL to 200 μg/mL, stock concentration 10 mg/mL) of fine paste of *L. alsinoides* and allowed to react at room temperature for 20 min in dark and absorbance was measured at 517 nm. 3 mL of DPPH was taken as control and Ascorbic acid (10 mg/mL DMSO) was used as the standard [19].

$$\text{Percentage of inhibition} = \text{Absorbance of}\left[\frac{\text{control} - \text{test}}{\text{control}} \times 100\right]$$

2.9. Preliminary phytochemical analysis

The physical and physicochemical parameters such as moisture content, volatile oil, ash values, fiber content, sugar content, extractive values and qualitative analysis for the presence of phytoconstituents were assessed as per the standard procedure mentioned in API [11]. Three samples were evaluated thrice for each test. The mean values were taken and expressed as mean ± S.E.

2.10. Statistical analysis

The serum biochemical parameters were presented as mean and standard error. One-way analysis of variance (ANOVA) was used and Tukey's multiple comparison test was done for determining the statistical significance. A probability value of <0.05 was considered as significant.

3. Results

3.1. Acute toxicity study

Throughout the observation period neither incidence of mortality nor animals found in a moribund condition were recorded. Body weight and other factors for toxicity evaluation were observed as normal. Histopathological examination showed normal architecture of liver, kidney and spleen (Fig. 2A, B, C). These results imply that *kalka* preparation (fine paste) of *L. alsinoides* Lam. is safe even at a dose of 2500 mg/kg, in tested rats.

3.2. Gross observations in hepatoprotective study

All the animals were seen healthy and did not show any deviation from normal activities or behavior. The liver collected from Group I was bright red in color and Group II was pale red in color and was slightly enlarged in size. The livers collected from Group III, to Group VI were enlarged and red in color.

3.3. Serum biochemical parameters

Table 1 shows summarized results of biochemical parameters collected from all groups. Administration of CCl_4 to the rats of Group II showed an obvious increase ($p < 0.01$) in AST, ALT, ALP, total bilirubin and total cholesterol and decline in serum albumin and total protein when compared with control group. Animals in Group III showed significant reduction ($p < 0.01$) in all parameters and increase in serum albumin and total protein on comparison with group II. The oral administration of *kalka* preparation (fine paste) of *L. alsinoides* Lam. (Group IV–VI) prevented CCl_4 induced increase in AST, ALT, total bilirubin ALP and total cholesterol and decrease in serum albumin and total protein levels compared with animals in Group II. In AST and ALT values, all *L. alsinoides* treated groups showed reduction, but significant effect was seen only in Group VI. In ALP, serum total bilirubin and total cholesterol values

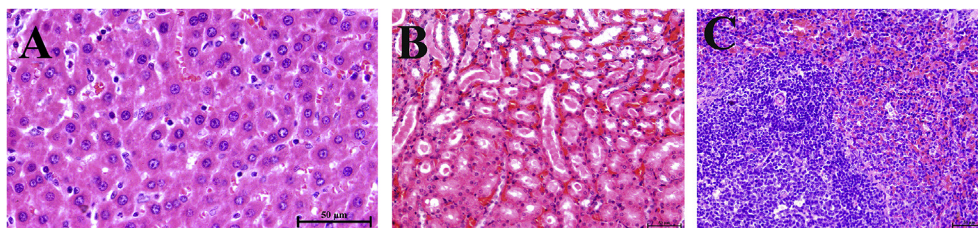

Fig. 2. Photomicrographs of Hematoxylin and Eosin stained histological sections of rat: (A) liver (B) Kidney (C) Spleen.

all *L. alsinoides* treated groups showed significant reduction and pronounced effect was seen in animals of Group VI. All the *L. alsinoides* treated groups showed significant increase in total protein and albumin values when compared with Group II.When the *L. alsinoides* treated groups were compared each other, pronounced effect was seen in Group VI (2.16 g/kg) similar to Silymarin group (Group III). No significant difference was seen on comparison of Group VI with Group III.

3.4. Histomorphological observations

Mild sinusoidal dilatation and mild congestion in central vein were present in all liver sections. The liver of group II (CCl_4 treated rats) animals had intense centrilobular necrosis, dilatation of central veins, congestion of sinusoids and vacuolar degeneration of hepatocytes more in the central region. Mild to moderate degree of fatty changes were observed in the midzonal and periportal hepatocytes (Fig. 3B).

In Group III (Silymarin treated rats), there was mild vacuolar degeneration or necrosis. Occasional mitotic figures were also observed. The inflammatory changes and degeneration of hepatocytes were less when compared to group II (Fig. 3C). In animals treated with the *L. alsinoides* histopathological examination showed hepatic regeneration with scattered mitotic figures in the parenchyma and the extent of damage was minimal (Fig. 3D–F). The damages seen were restricted to mild-moderate fatty changes and vacuolar degeneration. In Group III, *L. alsinoides* treated rats at a dose of 0.54 g/kg showed occasional mitosis (about 1/HPF). Mild centrilobular hepatocyte degeneration, vacuolar degeneration and periportal lymphoid accumulation were seen. In **Group V**, *L. alsinoides* treated rats at a dose of 1.08 g/kg showed more mitosis (about 1/HPF). Few areas of necrosis and extensive areas of vacuolar degeneration were found. In **Group VI**, *L. alsinoides* treated rats at a dose of 2.16 g/kg showed mitosis about 1/HPF. Only spotty and focal necrosis was observed in the central area of hepatic lobule. Normal histological structure was almost preserved.

3.4.1. Percentage of mitosis

Mitosis was observed in all experimental groups except Group II. The percentage of mitosis was relatively high in animals of Group V (Fig. 4A).

3.4.2. Quantification of vacuolar degeneration and necrosis

The extents of degeneration and necrosis were higher in histology sections of liver in animals of Group II. In Group III, vacuolar degeneration was lowest with mild necrosis. In Group IV, mild vacuolar degeneration and necrosis were present. Mild or moderate vacuolar degeneration was observed in Groups V and VI, but necrosis was considerably reduced and nearly absent in Group VI (Fig. 4B,C).

3.5. In vitro anti-oxidant activity

3.5.1. Hydroxyl radical scavenging activity

Fine paste of *L. alsinoides* showed good antiradical activity by inhibiting hydroxyl radicals similar to Gallic acid. Maximum hydroxyl radical scavenging (61.55%) was observed at 2000 μg/mL and that of gallic acid (at 2000 μg/mL) is 61.55%. IC_{50} was found to be 1445.57 μg/mL (Fig. 5A).

3.5.2. Nitric oxide radical scavenging activity

Fine paste of *L. alsinoides* showed good antiradical activity by inhibiting nitric oxide radicals similar to Gallic acid. Maximum nitric oxide radical scavenging (80.77%) was observed at 2000 μg/mL and that of gallic acid (at 2000 μg/mL) is 62.98%. IC_{50} was found to be 730.82 μg/mL (Fig. 5B).

3.5.3. DPPH radical scavenging activity

Fine paste of *L. alsinoides* showed antiradical activity by inhibiting DPPH in a dose dependent manner. Maximum DPPH radical scavenging (48.07%). was observed at 200 μg/mL and the IC_{50} was found to be above 200 μg/mL (Fig. 5C).

Table 1
Summarized results of Biochemical parameters.

Groups	AST (U/L)	ALT (U/L)	ALP U/L)	Total bilirubin (mg/dL)	Total protein (mg/dL)	Albumin (mg/dL)	Total cholesterol (mg/dL)
I	$102.3 \pm 14.0^{a,c,d}$	$58.5 \pm 2.3^{a,c,d}$	102.0 ± 6.0^{a}	0.8 ± 0.0^{a}	$6.4 \pm 0.1^{a,d,e}$	$4.9 \pm 0.1^{a,b,d}$	$99.5 \pm 4.0^{a,b,c,d}$
II	$309.2 \pm 30.0^{f,i}$	$231.7 \pm 27.0^{f,i}$	$266.2 \pm 14.0^{f,g,h,i}$	$2.0 \pm 0.1^{f,g,h,i}$	$3.2 \pm 0.2^{f,g,h,i}$	$1.4 \pm 0.2^{f,g,h,i}$	$191.7 \pm 5.9^{f,h,i}$
III	$132.5 \pm 23.0^{j,k}$	$84.2 \pm 11.0^{j,k}$	110.7 ± 3.0	0.7 ± 0.0^{j}	$6.5 \pm 0.2^{k,l}$	$3.3 \pm 0.2^{j,l}$	132.5 ± 7.3^{j}
IV	280.7 ± 28.0	177.8 ± 27.0	143.0 ± 19.0	$1.1 \pm 0.2^{m,n}$	$6.8 \pm 0.2^{m,n}$	4.8 ± 0.3^{m}	$183.8 \pm 8.1^{m,n}$
V	276.5 ± 24.0	199.8 ± 26.0^{o}	153.8 ± 13.0	0.7 ± 0.1	4.9 ± 0.4	3.7 ± 0.3	145.3 ± 4.6^{o}
VI	188.2 ± 28.0	97.8 ± 7.0	123.5 ± 36.0	0.7 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	119.2 ± 3.9

Data represent mean ± S.E (n = 6), Superscripts a, b, c, d, e, f, g, h, I, j, k, l, m, n, and o represent statistical significance (p value < 0.05) between groups I and II, I and III, I and IV, I and V, I and VI, II and III, II and IV, II and V, II and VI, III and IV, III and V, III and VI, IV and V, IV and VI, and V and VI respectively. The level of significance (p-value) for **AST**: a<0.0001, c = 0.0003, d = 0.0004, f = 0.0003, j = 0.0029, and k = 0.004. **ALT**: a<0.0001, c = 0.0021, d = 0.0002, f = 0.0001, i = 0.0005, j = 0.0228, k = 0.003 and o = 0.0108. **ALP**: a<0.0001, f < 0.0001, g = 0.0007, h = 0.0022 and i < 0.0001. **Bilirubin**: a<0.0001, f < 0.0001, g < 0.0001, h < 0.0001, i < 0.0001, j = 0.0321, m = 0.0321 and n = 0.0197. **Total protein**: a<0.000, d = 0.0003, e < 0.0001, f < 0.0001, g < 0.0001, h < 0.0001, i = 0.0018, k = 0.0001, l < 0.0001, m < 0.0001 and n < 0.0001. **Albumin**: a<0.0001, b < 0.0001, d = 0.001, f < 0.0001, g < 0.0001, h < 0.0001, i < 0.0001, j < 0.0001, l = 0.0007, m = 0.0048. **Total cholesterol**: a<0.0001, b = 0.0051, c < 0.0001, d = 0.001, f < 0.0001, h < 0.0001, i < 0.0001, j < 0.0001, m = 0.0009, n < 0.0001, o = 0.0392.

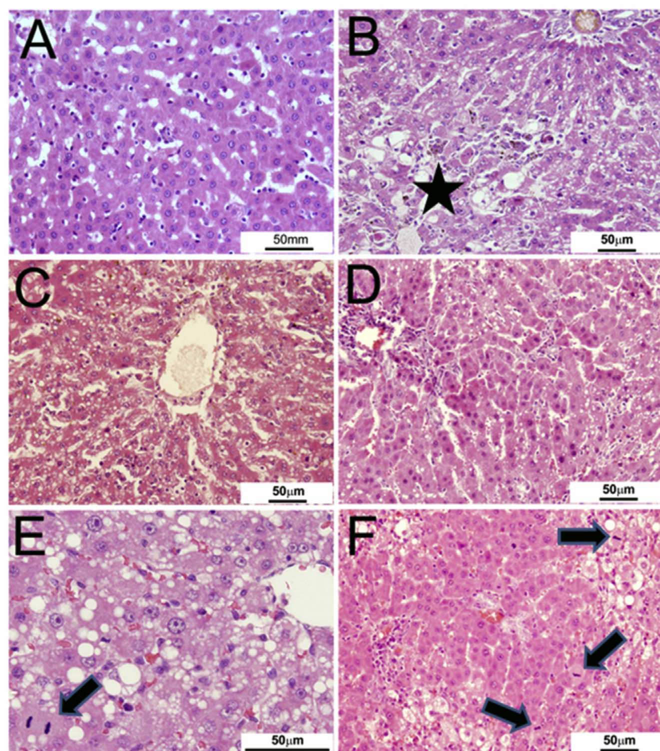

Fig. 3. Photomicrographs of Hematoxylin and Eosin-stained paraffin sections of rat liver from Group I (A) with histological features of liver and Group II (B) with severe centrilobular necrosis (black star) indicating induced liver damage after the administration of CCL_4. Following treatment with Silymarin (C) in Group III and *L. alsinoides* (D, E and F) in Groups IV (0.54 g/kg), V (1.08 g/kg) and VI (1.08 g/kg), there was remarkable recovery with variable extent of fatty degeneration (apparent as vacuoles in hepatocytes) and liver regeneration indicated by mitosis (arrow).

3.6. Preliminary phytochemical screening

The results of phytochemical analysis were depicted in Tables 2–4.

4. Discussion

The use of the plant *L. alsinoides* Lam. (Companulaceae family) for treating liver disorders has been recorded in *Hortus Malabaricus* and known to Ayurvedic practitioners since centuries [7]. However, the claim about its hepatoprotective effect has not been validated by any scientific data. This study examined if a *kalka* preparation of the plant has hepatoprotective effect in carbon tetrachloride-induced hepatotoxicity in Wistar albino rats. The candidate *kalka* formulation of the plant *L. alsinoides* Lam. containing all parts of the plant was made as per recommended procedure in API (Fig. 1C) and the *in vivo* hepatoprotective effect was evaluated in CCl_4 induced hepatotoxicity in albino rats. Carbon-tetrachloride is a commonly used chemical for inducing experimental liver toxicity in animal models [20,21] including studies intended for evaluation of drugs of Ayurvedic importance [13]. Animals in Group II (CCl_4 treated) had all hallmarks of hepatotoxicity. There was significant elevation in the biochemical values of ALT, AST, ALP and total bilirubin compared to normal animals in Group-I (Table 1).

Further, at necropsy of animals in Group II, the gross morphology of the liver was suggestive of widespread damage. At histopathology there was intense centrilobular necrosis, dilatation of central veins, congestion of sinusoids and vacuolar degeneration of hepatocytes. Mild to moderate degree of fatty changes were exhibited in the midzonal and periportal hepatocytes (Fig. 3B). The gross and histomorphological observations corroborated the blood chemistry data and were in consistence with the changes reported for hepatotoxicity in previous studies [13]. In animals treated with Silymarin (Group III), the rats did not show any perturbations in serum levels of ALT, AST, ALP, total bilirubin and total cholesterol and significant decrease in total protein and albumin ($p < 0.01$) and their liver was grossly red colored. At histopathology, mild necrosis and vacuolar degeneration were seen (Fig. 3C) but the extent of the lesions were less compared with Group II (Fig. 4B,C). The observations very strongly indicated that animal experimental protocols used for producing hepatotoxicity in this study are valid. Animals treated with the *kalka* (fine paste) preparation (Group IV, Group V and Group VI) under the study did not show elevation in biochemical parameters as in animals of Group III (Table 1). They had significantly reduced serum total bilirubin, ALP, and total cholesterol values and maintained total protein and albumin values when compared with CCl_4 treated rats. Moreover, the animals in Group VI had significant reduction in AST and ALT levels. Indeed, the animals in Group VI had serum biochemistry similar to the animals treated with Silymarin in Group III (Table 1). The results largely indicated that oral administration of the *L. alsinoides* safeguarded the CCl_4 in toxicatedanimals from liver damage On the other hand, among *L. alsinoides* treated groups, pronounced effect was seen in Group VI rather than in Group IV and Group V. Therefore there is a need for further investigation for identifying the most therapeutically effective dose.

The semi-quantitative data collected during histopathological examination (Fig. 4) provided more objective data in support of the claim that the *L. alsinoides* had hepatoprotective effect. The presence of mitosis indicated a possible regenerative response [22]. The percentage of mitosis was highest in Group V (Fig. 4A) indicating its higher regenerative efficiency. In all *L. alsinoides* treated groups, hepatic regeneration was evident with scattered mitotic figures in the parenchyma and the extent of damage was minimal (Fig. 3D–F). The lesions were predominantly fatty changes and vacuolar degeneration, which were reversible responses [23]. However,

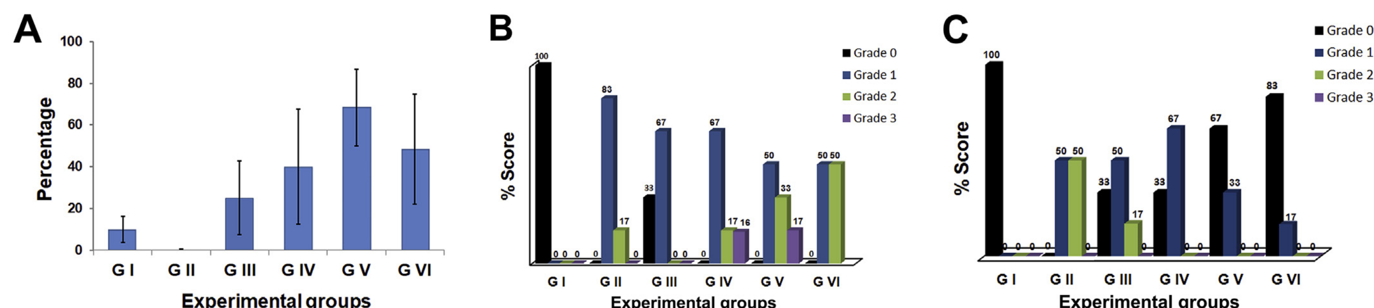

Fig. 4. Comparative Bar Diagram showing (A) percentage of mitosis (B) Grading of vacuolar degeneration (C) Grading of necrosis.

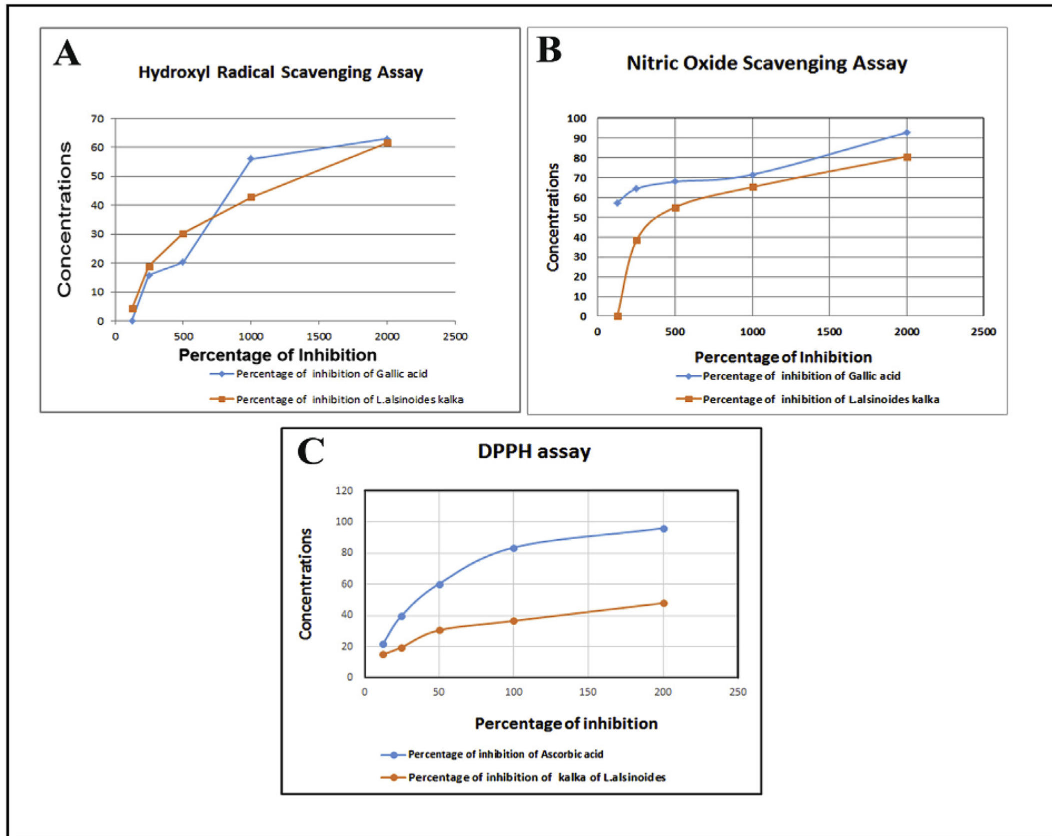

Fig. 5. Graphical representation of *in-vitro* anti-oxidant activity of fine paste of *L. alsinoides* Hydroxyl radical scavenging assay (A), Nitric oxide scavenging assay (B) and DPPH assay (C).

Table 2
physical and physicochemical parameters of *L. alsinoides* Lam. Data represents mean ± S.E (n = 3).

Sl No.	Parameters	Values (in percentage)
1	Moisture content	10
2	Volatile oil	Nil
3	Foreign matter	Nil
4	Total ash	17.23 ± 1.56
5	Acid insoluble ash	1.58 ± 0.28
6	Water insoluble ash	2.39 ± 1.39
7	Water soluble extractive	18.46 ± 0.53
8	Alcohol soluble extractive	6.69 ± 1.02
9	Fiber content	18.33 ± 1.24
10	Total sugar	5.22 ± 1.06
11	Reducing sugar	3.35 ± 0.82

Table 3
Qualitative analysis of different extracts of *L. alsinoides* Lam. (n = 3).

Phytoconstituents	Pet ether extract	Cyclohexane extract	Acetone extract	Methanol extract
Alkaloids	+	+	+	++
Sterol	++	+	+	+
Phenol	−	−	+	+
Flavonoid	−	−	−	−

necrosis was also present, but considerably reduced in all *L. alsinoides* treated groups and the lesions were very minimal in Group VI (Fig. 4C). On the other hand, the liver specimens from Group III showed mild vacuolar degeneration and necrosis. Thus the data indicates the hepatoprotective effect of *L. alsinoides* is on a par with Silymarin. The data collected in this study supported the claim that *L. alsinoides* had hepatoprotective effect. Further the results of biochemistry and histopathology suggested that the animals in Group VI which received dose 2.16 g/kg enjoyed the maximum hepatoprotective effect. It is important to note that the posology for treating the diseased rats in Group IV, Group V and Group VI was chosen based on *Sarngadhara Samhita* [15]. According to this, the dose of *kalka* preparations (fine paste) is 12 g/day for man. Based on human to rat dose conversion table [16] the expected therapeutic dosage (per os) for rats is 1.08 g/kg and is given to Group V. Its half dose (0.54 g/kg) and double dose (2.16 g/kg) were given to animals in group IV and Group VI respectively. However, maximum hepatoprotective activity appeared to have occurred in animals Group VI, which were treated with 2.16 g/kg. Further studies are necessary to determine the potential of still higher doses. Nevertheless, the data presented here strongly suggested that *kalka* formulation (fine paste) as per Ayurvedic Pharmacopoeia of India has hepatoprotective effect in Wistar albino rats.

The anti-oxidant activity of fine paste of *L. alsinoides* was evaluated by hydroxyl radical, nitric oxide and DPPH assays and *L. alsinoides* showed anti-oxidant activity in all the three methods, but best scavenging potential was observed with hydroxyl radicals (Fig. 5). Preliminary phytochemical screening revealed the presence of steroids, alkaloids, phenol and tannins in the plant (Tables 2–4). Further investigations are needed for the chemical characterization of the plant.

The mechanism of the hepatoprotective action of the plant was uncertain from this study but may be related to the capacity of the plant derivatives to prevent lipid peroxidation by its free radical scavenging activity in the liver as recorded for other plant

Table 4
Tests used for qualitative analysis of *L. alsinoides* Lam. (n = 3).

Phytoconstituents	Tests used	Results
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Glycosides	Keller–killani test	–
	Borntrager's test	–
	Legal test	–
Phenol	Ferric chloride test	+
Flavonoid	Shinoda test	–
Sterols	Salkowaski reaction	+
	Liebermann's test	+
Tannins	Ferric chloride reagent	+
	Lead acetate test	+
	Potassium dichromate test	+
Sugars	Fehling solution test	+
Proteins	Biuret test	+
	Xanthoproteic test	+
Saponins	Foam test	+

derivatives [24–26]. Nevertheless, the results of biochemical investigation and histomorphology suggested minimal liver damage.

Till this date no toxicity has been described for *L. alsinoides* Lam. However safety of the drug has to be proved as per the guidelines of World Health Organization (WHO) in the evaluation of traditional medicine. Hence an acute toxicity limit test was conducted as per OECD guidelines 425 [27]. Since no mortality and morbidity of the experimental animals were observed, and gross pathological and histopathological examination revealed no abnormality, it can be assumed that drug does not possess any acute toxicity up to 2500 mg/kg and the median lethal dose and maximum tolerated dose would be more than this dose.

Moreover, these protective effects over the damages caused in liver parenchyma by other factors like infectious agents (hepatitis viruses) have to be investigated. Despite these limitations, the study justified the traditional practice of *Cheriya manganari* for the management of liver diseases. It is certainly a promising source of a hepatoprotective drug.

5. Conclusions

It was concluded that a *kalka* formulation prepared of the whole plant *L. alsinoides* Lam. (Lobeliaceae) as per Ayurvedic Pharmacopoeia of India has significant liver protective efficacy in hepatotoxicity produced by Carbon-tetra-chloride in rats. Pronounced effect was seen at a dose of 2.16 g/kg in rats, under the present experimental conditions that is comparable to a human dose of 24 g/day. The results strongly justified the traditional use of *L. alsinoides* Lam. Certainly it is an acceptable Ayurvedic formulation for treating hepatobiliary diseases.

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Conflict of interest

None

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