Evaluation of hepatoprotective activity of alcoholic and aqueous extracts of *Selaginella lepidophylla*

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**PEER REVIEW**

Peer reviewer
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**ABSTRACT**

**Objective:** To screen *Selaginella lepidophylla* (*S. lepidophylla*) that are used in traditional medicine for their claimed hepatoprotective properties.

**Methods:** Alcoholic and aqueous extracts of *S. lepidophylla* were evaluated for their hepatoprotective activity using CCl₄ and paracetamol induced acute hepatic injury model.

**Results:** Treatment with CCl₄ and paracetamol significantly increased liver weight and volume compared to the normal group. Pretreatment with silymarin alcoholic and aqueous extracts significantly prevent increase in liver weight and volume.

**Conclusions:** From the present experimental study it can be concluded that alcoholic and aqueous extract of *S. lepidophylla* exhibited significant hepatoprotective activity against CCl₄ and paracetamol induced hepatotoxicity in rats, as result showed in physical, biochemical and histopathological parameters.

**KEYWORDS**
Hepatoprotective, *Selaginella lepidophylla*, CCl₄, Paracetamol

**1. Introduction**

Hepatic fibrosis represents the response of the liver to diverse chronic insults such as parasitic disease, chronic viral infection (hepatitis B and C), immunologic attack (autoimmune hepatitis), hereditary metal overload, toxic damage, etc.[1]. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue glutathione levels. In addition serum levels of many biochemical markers like serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated[2]. Hepatic cells are involved in a variety of metabolic events; therefore the establishment of liver protective/therapeutic agents is of paramount importance in the protection from liver damage. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. The previous studies have shown that hepatoprotective effects are associated with phytoextracts/phytocompounds rich in natural antioxidants[3–7]. Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin–induced liver damage[8,9]. In addition, recent researches on hepatoprotective medicinal plants as a major indicator of the screening systems can trigger the safety evaluation in the early phase of drug discovery because most of the toxic compounds are metabolized in liver[10–13].

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Selaginellas have similar morphological characteristics, particularly having heterospore form and loose strobili; and are classified as one genus and one family. However, individual species has high morphological variation caused by different edaphic and climatic factors. Genetic studies indicate high polymorphism among Selaginella species. Selaginella had been used as complementary and alternative medicines on postpartum, menstrual disorder, wound, etc. Bioflavonoid, the main secondary metabolites give this benefit and are especially used as anti oxidant, anti-inflammatory, and anti cancer in modern pharmaceutical industry\cite{14}. Therefore, in view of conforming the effects on hepatitis, various organic extracts from the whole plant of Selaginella lepidophylla (S. lepidophylla) were prepared and their hepatoprotective effects were evaluated in experimental models of liver injury induced by CCl₄ and paracetamol in rats.

2. Materials and methods

2.1. Collection of plant materials

Fresh whole plants of S. lepidophylla were collected during June–July 2011 from the Amarkantak hills, India. The Identification and authentication of the plant was carried out by Prof. P. Jayaraman, National Institute Of Herbal Science, Plant Anatomy Research Centre (PARC), 4, 2nd Street, Sakthi Nagar, West Tambaram, Chennai, India. (Reg. No. of the certificate PARC/2011/990).

2.2. Preparation of extracts (solvent- alcohol and water)

Plants were dried in shade. The dried plant material was then subjected to size reduction to obtain coarse powder using a grinding mill. Powdered material were Soxhlet extracted successively with increasing order of polarity. The method is described as the continuous extraction. The process is continued until all the soluble constituents get separated. Extraction was continued at the temperature of 50 °C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40 °C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40 °C till clear solvent was observed in siphon tube. Concentrated extract was dried at 40 °C in hot air oven. Dried extract was packed in an air tight container and alcoholic extract of S. lepidophylla was also extracted\cite{15}.

2.3. Animals

Albino Wistar rats were obtained from animal house of School of Pharmacy, Chouksey Engineering College, Bilaspur, India. The experiment was conducted as per the permission of Institutional Animal Ethical Committee (IAEC) of School of Pharmacy, Chouksey Engineering College (Regd No. 1275/ac/09/CPCSEA). All conditions were maintained according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms. The animals of either sex were selected randomLy of uniform weight (120±5) g from animal house. The room temperature was maintained at (22±2) °C with food (Lipton India Ltd. pellets) and water ad libitum. The animals were transferred to the laboratory at least 1 h before the start of the experiment. The experiments were performed during day (08:00 a.m.–16:00 p.m.). The Institutional Animal Ethical Committee approved to the study protocol.

2.4. Behavioral tests

All behavioral tests were conducted in 4–months old male albino Wistar rats. The animals were housed under standard laboratory conditions as per the CPCSEA guidelines. All experiments were approved by Institutional Animal Ethics Committee. After 2 weeks of acclimatization, all animals were subjected to tests for locomotion, exploration and anxiety, followed by the learned helplessness procedure. Animals were shifted to the laboratory (experimental room) at least 60 min before experiment. All behavioral tests were conducted during the light cycle, i.e. during the animals’ active phase.

2.5. Acute toxicity study

The acute oral toxicity study was performed according to the Office of Prevention, Pesticides and Toxic Substance (OPPTS) guidelines\cite{16}.

2.6. Selection of dose

For the assessment of hepatoprotective activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose during acute toxicity studies (250 and 500 mg/kg body weight).\cite{17}

2.7. Vehicle and standard

Distilled water and Tween 80 (5%) were used as vehicle for preparing the suspension of various test doses of extracts. Silymarin (50 mg/kg body weight) was used as standard drug.

2.8. Animal group classification

Wistar rats weighing 200–250 g were divided into seven groups consisting of six animals in each group. Group 1 received distilled water (6 mL/kg, p.o.) for four days. Group 2 were treated with vehicle (2% acacia, 1 mL/kg, p.o.) for four days. Group 3 received silymarin (50 mg/kg body weight, p.o.) for four days. Group 4 and 5 were pretreated with alcoholic extract of S. lepidophylla 250 and 500 mg/kg body weight respectively for four days. Group 6 and 7 were pretreated with aqueous extract of S. lepidophylla 250 and 500 mg/kg body weight respectively for four days. The same sets of groups were used for CCl₄ and paracetamol induced liver injury liver damage. Separate sets of rats were used for each experiments.

2.9. Carbon tetrachloride induced acute hepatotoxicity

Male albino adult rats weighing 200–250 g body weight were divided into 7 groups and each group consisting of six rats. Food was withdrawn 12 h before CCl₄ administration to enhance the acute liver damage in animal. Groups 2, 3, 4, 5, 6 and 7...
received a single dose of CCl₄ (2 mL/kg, p.o.) diluted with liquid paraffin (1:1) on the fourth day after 1 h of extract treatment and sacrificed 24 h after administration of CCl₄. The animals were euthanized and blood samples were collected by retro orbital method and serum was used for estimation of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and direct bilirubin. The liver was washed by normal saline, blotted with filter paper and weighed immediately and liver sample were preserved in 10% formalin for histopathological studies.

2.10. Paracetamol induced hepatotoxicity

Male albino adult rats weighing 200–250 g were grouped as mentioned previously. Food was withdrawn 16 h before paracetamol administration to enhance the acute liver toxicity. Groups 2, 3, 4, 5, 6 and 7 received a single dose of paracetamol (450 mg/kg body weight, i.p.) diluted with propylene glycol (12.5% solution) on the fourth day after 1 h of extract treatment and sacrificed 6 h after administration of paracetamol. After 6 h all the animal were euthanized and blood samples were collected by retro orbital method and serum was used for estimation of AST, ALT, ALP and direct bilirubin. The liver was washed by normal saline, blotted with filter paper and weighed immediately and liver sample were preserved in 10% formalin for histopathological studies.

2.11. Statistical analysis

The data obtained by the various parameters was statistically evaluated by One way analysis of variance (ANOVA) followed by Dunnett’s test. The mean values±SEM were calculated for each parameter[18].

3. Result

3.1. Acute oral toxicity study

Different doses of alcoholic and aqueous extract were screened for their oral toxicity. No mortality was recorded till 5000 mg/kg body weight with alcoholic and aqueous extract, hence the extract was found to be safe upto the dose levels of 5000 mg/kg body weight.

3.2. Acute hepatic injury

3.2.1. Carbon tetrachloride induced acute hepatic injury

3.2.1.1. Liver weight and volume

Treatment with CCl₄ significantly increased the liver weight and volume compared to the normal group. Pretreated with silymarin, alcoholic and aqueous extracts of *S. lepidophylla* significantly prevent the increase in liver weight and volume induced by CCl₄ (Table 1).

| Treatment                   | Mean liver weight (g/100 g) | Mean liver volume (ml/100 g) |
|-----------------------------|-----------------------------|------------------------------|
| Distilled water             | 4.21±0.12                   | 4.45±0.12                    |
| 2% w/v acacia+CCl₄ (2 mL/kg, p.o.) | 4.41±0.14                   | 4.94±0.14                    |
| Silymarin (50 mg/kg, p.o.)+CCl₄ (2.0 mL/kg, p.o.) | 4.15±0.14                   | 5.05±0.12                    |
| ALSL (250 mg/kg, p.o.)+CCl₄ (2.0 mL/kg, p.o.) | 5.44±0.11                   | 4.04±0.12                    |
| ALSL (500 mg/kg, p.o.)+CCl₄ (2.0 mL/kg, p.o.) | 4.44±0.09                   | 4.59±0.14                    |
| AQSL (250 mg/kg, p.o.)+CCl₄ (2.0 mL/kg, p.o.) | 5.39±0.10                   | 5.94±0.20                    |
| AQSL (500 mg/kg, p.o.)+CCl₄ (2.0 mL/kg, p.o.) | 4.14±0.05                   | 4.42±0.12                    |

ALSL: Alcoholic extract of *S. lepidophylla*, AQSL: Aqueous extract of *S. lepidophylla*.

3.2.1.2. Serum biochemical parameters

CCl₄ (2 mL/kg, p.o.) administration resulted in significant elevation of AST, ALT, ALP and direct bilirubin compared to the normal group. Pretreated with silymarin, alcoholic and aqueous extracts of *S. lepidophylla* significantly prevented biochemical changes induced by CCl₄ (Table 2).

3.2.1.3. Histology

In normal animal, liver section showed normal hepatic cells with well preserved cytoplasm, prominent nucleolus and central vein (Figure 1A). In CCl₄ treated animals the section showed sever degree of damage liver, congestion, macro and micro–vesicular steatosis (Figure 1B). In alcoholic extracts of *S. lepidophylla* treated animals, liver section shows mild degree of liver damage, sinusoidal congestion, mild inflammation and mild degree of macro and micro–vesicular steatosis (Figure 1C). In aqueous extracts of *S. lepidophylla* treated animals, liver section shows moderate degree of liver damage, sinusoidal congestion, mild inflammation and moderate degree of macro and micro–vesicular steatosis (Figure 1D). In silymarin (50 mg/kg, p.o.) treated animals, liver section shows mild degree of liver damage, sinusoidal congestion, mild inflammation and milder degree of macro and micro–vesicular steatosis (Figure 1E).

![Figure 1](image_url)
3.2.2. Paracetamol induced acute hepatic injury

3.2.2.1. Liver weight and volume

Administration of paracetamol has produced a significantly increased in liver weight and volume. Rats pretreated with silymarin (50 mg/kg, p.o.), alcoholic extracts of S. lepidophylla (250 and 500 mg/kg, p.o.) and aqueous extracts of S. lepidophylla (250 and 500 mg/kg, p.o.) showed significantly decrease in liver weight and volume compared to toxic control group (Table 3).

3.2.2.2. Serum biochemical parameters:

Paracetamol (450 mg/kg, i.p.) administration resulted in significant elevation of the biochemical parameters like AST, ALT, ALP and direct bilirubin compared to the normal group. Pretreated with silymarin, alcoholic and aqueous extracts of S. lepidophylla significantly prevented the biochemical changes induced by paracetamol. The hepatoprotective effect offered by alcoholic extracts of S. lepidophylla (500 mg/kg, p.o.) was found to be significantly greater than aqueous extracts of S. lepidophylla (500 mg/kg, p.o.) and standard silymarin (50 mg/kg) group (Table 4).

3.2.2.3. Histology

In normal animal, liver section showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and central vein (Figure 2A). In paracetamol treated animals the section showed moderate degree of damage liver, showing periporal and lobular inflammation and mild congestion. The changes due to paracetamol are very mild when compared to CCl4 intoxication (Figure 2B). In alcoholic extracts of S. lepidophylla (500 mg/kg, p.o.) treated animals, liver section showing moderate inflammation and congestion (Figure 2C). In aqueous extracts of S. lepidophylla (500 mg/kg, p.o.) treated animals, liver section shows moderate degree of liver damage, congestion, mild inflammation and moderate degree of macro and micro–vesicular steatosis (Figure 2D). In silymarin (50 mg/kg, p.o.) treated animals, liver section shows mild inflammation and moderate congestion (Figure 2E).

Table 2

| Treatment | Serum biochemical parameters |
|-----------|-------------------------------|
|           | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | TBIL (mg/dL) |
| Distilled water | 84.730±0.120 | 55.740±0.664 | 138.350±0.212 | 0.518±0.001 |
| 2% w/v acacia+Paracetamol (2.0 mL/kg, p.o.) | 348.990±1.145 | 196.850±2.700 | 343.800±0.424 | 2.120±0.014 |
| Silymarin (50 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, p.o.) | 149.460±0.197 | 64.040±0.219 | 293.550±1.790 | 0.938±0.001 |
| ALSL (250 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, p.o.) | 110.780±0.113 | 102.250±0.777 | 288.50±2.494 | 0.915±0.021 |
| ALSL (500 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, p.o.) | 139.450±0.353 | 88.480±0.692 | 225.200±0.424 | 0.735±0.035 |
| AQSL (250 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, p.o.) | 129.700±0.282 | 179.350±0.212 | 273.500±0.424 | 0.940±0.014 |
| AQSL (500 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, p.o.) | 134.750±0.056 | 120.850±0.315 | 206.900±0.014 | 0.630±0.014 |

Table 3

| Treatment | Mean liver weight (g/100 g) | Mean liver volume (ml/100 g) |
|-----------|-----------------------------|-------------------------------|
| Distilled water | 2.2750±0.0077 | 4.680±0.023 |
| 2% w/v acacia+Paracetamol (2.0 mL/kg, p.o.) | 4.5300±0.0002 | 4.230±0.014 |
| Silymarin (50 mg/kg, p.o.)+Paracetamol (450 mg/kg, i.p.) | 2.4200±0.4500 | 3.130±0.160 |
| ALSL (250 mg/kg, p.o.)+Paracetamol (450 mg/kg, i.p.) | 6.050±0.0230 | 7.480±0.560 |
| ALSL (500 mg/kg, p.o.)+Paracetamol (450 mg/kg, i.p.) | 5.070±0.0094 | 4.160±0.072 |
| AQSL (250 mg/kg, p.o.)+Paracetamol (450 mg/kg, i.p.) | 5.8400±0.0580 | 5.860±0.135 |
| AQSL (500 mg/kg, p.o.)+Paracetamol (450 mg/kg, i.p.) | 4.8700±0.1670 | 4.420±0.012 |

Table 4

| Treatment | Serum biochemical parameters |
|-----------|-------------------------------|
|           | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | TBIL (mg/dL) |
| Distilled water | 94.720±0.077 | 45.270±0.560 | 110.40±0.13 | 0.214±0.0007 |
| 2% w/v acacia+Paracetamol (2.0 mL/kg, p.o.) | 445.800±0.197 | 156.800±1.490 | 253.80±1.34 | 1.050±0.0140 |
| Silymarin (50 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, i.p.) | 242.600±0.176 | 50.590±1.300 | 194.20±0.63 | 0.585±0.0070 |
| ALSL (250 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, i.p.) | 211.700±0.141 | 94.800±1.120 | 224.20±0.63 | 0.520±0.0140 |
| ALSL (500 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, i.p.) | 256.20±0.586 | 59.770±0.480 | 145.60±1.61 | 0.440±0.0420 |
| AQSL (250 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, i.p.) | 219.50±0.070 | 119.50±0.070 | 248.20±0.87 | 0.950±0.0400 |
| AQSL (500 mg/kg, p.o.)+Paracetamol (2.0 mL/kg, i.p.) | 254.71±0.056 | 94.14±0.690 | 209.60±0.04 | 0.440±0.0400 |
The treatment of liver diseases[20]. In the present study CCl4 and most of the herbal formulations can not be recommended for agents, CCl4, thioacetamide cure diseases. However, most of these products lack scientific various toxic chemicals (certain anti-biotic, chemotherapeutic effect of alcoholic and aqueous extracts of silymarin in acute paracetamol model (haematoxylin and eosin, 400伊). Liver is a vital organ that plays a major role in metabolism and drug induced liver damage. The present study was also methodological scientific evaluation for their safety and efficacy by subjective to vigorous pre–clinical studies followed by clinical trials to unravel the mysteries hidden in the plants. This approach will help exploring the real therapeutic value of these natural pharmacotherapeutic agents and standardized the dosage regimen on evidence–based findings[20]. Many herbs are in the market to support health, relieve symptoms and cure diseases. However, most of these products lack scientific pharmacological validation. In experimental hepatotoxicity models in laboratory or higher animals, several herbs exerted hepatoprotective/curative effects that warrants their clinical testing. Due to lack of scientific–based pharmacological data, most of the herbal formulations can not be recommended for the treatment of liver diseases[20]. In the present study CCl4 and paracetamol were selected as a hepatotoxicant to induced liver damage. The primary objective of this study was to assess the hepatoprotective activity on S. lepidophylla against chemically and drug induced liver damage. The present study was also carried out using two different model of liver to determine the effect of alcoholic and aqueous extracts of S. lepidophylla.

Carbon tetrachloride is very good hepatotoxin. Single doses lead to necrosis and steatosis after a very short latent time. Within a couple of minutes there is an injury towards the endoplasmic reticulum, which leads to functional defects of hepatocytes and multiple biochemical manifestation of hepatic injury. Steatosis develops because of movement of fat from the cell is blocked by disruption of mechanism for coupling triglycerides to the appropriate apoprotein to form the lipoprotein carrier molecule. During hepatic damage, enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in enhanced concentration. Single dose (2 mL/kg, p.o.) of CCl4 significantly enhanced all serum enzymes where as the S. lepidophylla extracts pretreated animal had significantly reduced AST, ALT, ALP and total bilirubin levels, indicating their hepatoprotective effect against CCl4 induced liver damage. Water is retained in cytoplasm of hepatocytes leading to enhancement of liver cells, resulting in increases total liver mass and volume observed in the present study. The CCl4 induced increases in total liver weight and volume were prevented by pretreatment with S. lepidophylla extracts, thus indicating a hepatoprotective effect. Continuous administration of CCl4 can lead to cirrhosis. In CCl4 treated animals the liver section showed degree of liver damage and was prevented by pretreatment with S. lepidophylla extracts, therefore indicating a hepatoprotective effect.

Paracetamol is well renowned analgesic and antipyretic agent which produces hepatotoxicity in higher dose. Paracetamol damages liver by covalent binding of its toxic metabolic N–acetyl–p–benzoquinoneimine to sulphhydryl group of proteins resulting in lipid per–oxidation induced by decreases in glutathione in the liver. In the present study in animals pretreated with S. lepidophylla extracts and silymarin, the total liver weight and volume and serum AST, ALT, ALP and total bilirubin levels were significantly lowered. Histopathological observation postulated that in experimental animals pretreated with alcoholic and aqueous extracts of S. lepidophylla and silymarin showed similar to that of normal liver. From the result it may be concluded that the hepatotoxicity activity of the extracts were in the ordered of alcoholic extract of S. lepidophylla, S. lepidophylla extract, S. lepidophylla (500 mg/kg, p.o.), Silymarin (50 mg/kg, p.o.), aqueous extract of S. lepidophylla (500 mg/kg, p.o.). So, the result of current study clearly revealed the various biochemical parameters (AST, ALT, ALP and total bilirubin, physicals (liver weight and volume) and histopathological alterations produced by CCl4 and paracetamol in the serum and tissue were reserved significantly by the pretreatment of plant extracts of S. lepidophylla.

In the present experimental study it can be concluded that alcoholic and aqueous extracts of S. lepidophylla had exhibited significant hepatoprotective activity against CCl4, and paracetamol induced hepatotoxicity in rats, as result showed in physical, biochemical and histopathological examination. The phenolic compounds and tannins were present in alcoholic and aqueous extracts of S. lepidophylla and it was reported that these two phytoconstituent were responsible for their hepatoprotective and antioxidant effect. These active principles can be accounted for hepatoprotective effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The present article approaches was to find out and screen *S. lepidophylla* that is used in traditional medicine for its claimed hepatoprotective properties.

Research frontiers

Studies are being performed in order to determine hepatoprotective properties of *S. lepidophylla* effects that are associated with phytoextracts/phytocompounds rich in natural antioxidants.

Related reports

There have many researches on plant extrat exerting hepatoprotective effect previously.

Innovations & breakthroughs

Many bioactive compounds and extracts from plants have been investigated for hepatoprotective and antioxidant effects against hepatotoxin--induced liver damage. In addition, recent research on hepatoprotective medicinal plants as a major indicator of the screening systems can trigger the safety evaluation in the early phase of drug discovery because most of the toxic compounds are metabolized in liver.

Applications

Popularity of herbal remedies is increasing globally and at least one quarter of patients with liver diseases use ethnomedicines. More efforts need to be directed towards methodological scientific evaluation for their safety and efficacy by subjecting to rigorous preclinical studies followed by clinical trials to unravel the mysteries hidden in the plants. This approach will help exploring the real therapeutic value of these natural pharmacotherapeutic agents and standardizing the dosage regimen on evidence-based findings.

Peer review

This is a good study in which the authors evaluated the hepatoprotective of *S. lepidophylla* and antioxidant effects against hepatotoxin--induced liver damage. The results are interesting and suggested that *S. lepidophylla* is a hepatoprotective medicinal plant.

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