

## Effect of *Lindernia ciliata* (Colsm.) Pennell. against Ethanol Induced Oxidative Damage in HEPG2 Cells

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### ABSTRACT

Objective: The study was aimed to assess the in-vitro hepatoprotective activity of methanolic extract of whole plant of *Lindernia ciliata* (Colsm.) Pennell. (LCME) of family Scrophulariaceae against ethanol induced cytotoxicity in HepG2 cell lines. Methods: The cytotoxicity study was conducted for the extract, LCME using MTT assay to determine the CTC 50 value. Based on the doses 50, 100 and 200 µg/ml were selected for the hepatoprotective study in HepG2 cell lines. The toxicity was induced by using ethanol (100mM). The in-vitro hepatoprotective activity of the extract was assessed based on the changes in the level of biochemical parameters such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH). Results: The extract, LCME has shown a significant cytoprotective activity with maximum protection and percentage cell viability (69.36%) at 100µg/mL. Conclusion: The study revealed that the extract, LCME has significant in-vitro hepatoprotective activity against ethanol induced cytotoxicity in Hep G2 cell lines.

**Key words:** Ethanol, HepG2, Hepatoprotective activity, *Lindernia ciliata*, MTT.

### INTRODUCTION

Among the various diseases, chronic liver diseases stand one of the serious health problems world wide<sup>1</sup>. Herbal medicines are believed to be much safer and proved elixir in the treatment of various ailments such as diabetes, liver disorders, CNS disorders etc<sup>2</sup>. According to World Health Organization (WHO) about 25% of prescribed drugs worldwide are derived from plants<sup>3</sup>. However, only a small proportion of hepatoprotective plants used in traditional medicine are pharmacologically evaluated for their safety and efficacy and many are yet to be investigated<sup>4</sup>.

The plant *Lindernia ciliata* (Colsm.) Pennell. of family Scrophulariaceae is a low growing, stoloniferous, mat-forming, annual, herb from 0.13 - 0.20m high. In India it was found as an insignificant weed, mainly in rice fields<sup>5</sup>. Traditionally it is used as a remedy for gonorrhoea, jaundice, urinary disturbances, bronchitis, headache, liver complaints, spleen diseases, constipation, fever, loss of appetite, asthma, cough, skin diseases<sup>6</sup>. There are no reports on the scientific validation of its traditional medicinal claim. In view of this, an attempt has been made in the present investigation to validate its traditional claim in the treatment of liver disorders by using in-vitro Hep G2 cell line model which are suitable for in-vitro model system to study the human liver diseases that are caused by xenobiotic metabolism and other chemicals that cause toxicity to liver<sup>7</sup>. The objective of the present work is to investigate the methanolic extract of whole plant of *Lindernia ciliata* (LCME) for its in-vitro hepatoprotective activity against ethanol

using HepG2 cell lines.

### MATERIALS AND METHODS

#### Preparation of the plant extract

The whole plant of *Lindernia ciliata* was collected in the month of August 2015, from rice fields of Bayyaram, Warangal district, Telangana state, India, after its authentication by Prof. V.S. Raju, Taxonomist, Kakatiya University, Warangal. The material was washed under tap water and shade dried, coarsely powdered (1 kg) and macerated with methanol in a round bottom flask for 7 days with intermittent stirring and filtered after seven days and concentrated under reduced pressure to yield a dark green semi solid mass. The percentage yield of the extract was found to be 7.4%.

#### Cell lines, Drugs and Chemicals

HepG2 Cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune India. The drugs and chemicals were purchased from various companies and the details are as follows: Dulbecco's modified eagles medium (DMEM), Silymarin- Sigma Aldrich, Spruce Street, St. Louis, China; Biochemical kits - Merck Specialties Private Limited, Mumbai, India; Fetal bovine serum (FBS) was purchased from Hi-media laboratories, Mumbai, India; Ethanol- Changshu yangyuan chemicals, China. All other chemicals and solvents used were of analytical grade.

#### Phytochemical analysis

The methanolic extract of *Lindernia ciliata* (LCME), was subjected to chemical tests for detection of various

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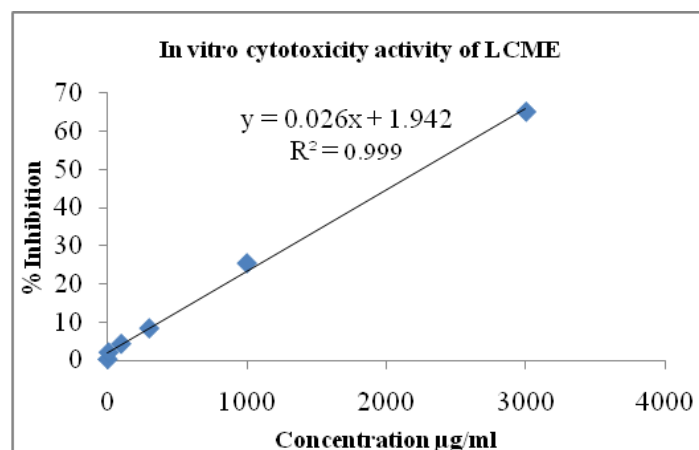


Figure 1: In vitro cytotoxic activity of LCME

phytoconstituents such as saponins, steroid/triterpenoidal, flavonoidal compounds and their glycosides, alkaloids and tannins<sup>8</sup>.

#### Determination of in-vitro cytotoxic activity

The CTC 50 (50% cytotoxic concentration) was determined by estimating mitochondrial synthesis using tetrazolium assay<sup>9</sup>. HepG2 cells ( $5.0 \times 10^3$  cells/ well) were maintained in 96 well culture plate for 72 h in presence of 100 µL of LCME at the concentrations of 10, 30, 100, 300, 1000 and 3000 µg/mL. At the end of incubation period, the drug solutions in the wells were discarded and 50 µL of MTT prepared in Modified Eagle's Medium (MEM) without phenol red (MEM-PR) was added in each well. The plates were gently shaken and incubated for 3h at 37°C in 5% CO<sub>2</sub> atmosphere. After 3h, the supernatant was removed. Later on, 50 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan followed by 30 min incubation at room temperature with constant shaking. Absorbance or optical density (OD) was read at 540nm using microplate reader (Bio-Tek Instruments, Inc., Winooski,VT). The percentage growth inhibition was calculated using the following formula:

$$\% \text{ Growth inhibition} = \frac{(\text{Mean OD of Normal control} - \text{Mean OD of test group})}{\text{Mean OD of Normal control}} \times 100$$

A dose–response curve was generated using % growth inhibition on Y axis and the extract concentration (µg/mL) on X-axis. The CTC 50 value is calculated from dose–response curve.

#### Assessment of hepatoprotective activity of LCME in-vitro

The hepatoprotective activity of LCME was evaluated using well maintained HepG2 cells. Ethanol was used as hepatotoxicant and Silymarin was used as a standard positive control. The toxic concentration of ethanol taken was 100 mM. The choice of concentrations of LCME and standard was based on the results of the MTT assay. The experimental groups are as follows:

##### Group I (Control)

Normal control: The cells were treated with 100 µL of serum free culture medium for 24h.

DMSO (Dimethyl sulphoxide) control: The cells were treated with 100 µL of serum free culture medium containing DMSO (0.3% v/v) for 24h.

Silymarin control: The cells were treated with 100 µL of serum free culture medium containing Silymarin (100 µg/mL) for 24h.

LCME control: The cells were treated with 100 µL of serum free culture medium containing LCME (200 µg/mL) for 24h.

Group II (Toxin treatment): The cells were treated with 100 µL of serum free culture medium containing 100 mM ethanol for 24 h.

Group III (Silymarin treatment): The cells were treated with 100 µL of serum free culture medium containing 100 mM ethanol with Silymarin at a concentration of 50 and 100 µg/mL for 24 h.

Group IV (LCME treatment): The cells were treated with 100 µL of serum free culture medium containing 100 mM ethanol with LCME at a concentration of 50, 100 and 200 µg/mL for 24h. Later, the cell supernatant was collected and the cell viability, AST, ALT and LDH leakage assays were performed for all groups according to the standard method<sup>7</sup> using Ecoline diagnostic kits. The change in the level of biochemical parameters was used to assess the hepatoprotective activity.

#### Statistical analysis

The data obtained were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test using Graph pad prism version 4 (Graph Pad Software, La Jolla California USA).

## RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the methanolic extract of whole plant of *Lindernia ciliata* (LCME) revealed that it contains chemical constituents of pharmacological significance such as steroidal/triterpenoidal and flavonoidal glycosides, saponins and phenolic compounds.

#### In-vitro cytotoxic activity

The results are shown in fig. 1. The CTC 50 value of LCME was found to be 2249.87 µg/mL. In MTT assay the cell viability was >85% up to a concentration of 1000 µg/mL. Therefore, the extract concentrations of 50, 100 and 200 µg/mL were selected to evaluate the cytoprotective activity against ethanol induced cell damage.

#### In-vitro hepatoprotective activity of the extract, LCME

Table 1: In-vitro Hepatoprotective activity of LCME using HepG2 cell lines.

Groups	%Cell viability	LDH	ALT	AST
Group I (Control)				
1. Normal control:	98.65±1.1	128.33±4.14 <sup>a</sup>	8.25±1.24 <sup>a</sup>	10.51±1.43 <sup>a</sup>
2. DMSO control (0.1% v/v)	94.54±1.04	141.11±3.34 <sup>a</sup>	9.33±1.24 <sup>a</sup>	13.14±1.02 <sup>a</sup>
3. Silymarin control (100 µg/mL)	96.12±1.02	138.36±4.16 <sup>a</sup>	8.93±1.25 <sup>a</sup>	12.18±1.11 <sup>a</sup>
4. LCME control (200 µg/mL)	95.23±1.08	140.31±3.76 <sup>a</sup>	9.12±2.11 <sup>a</sup>	13.06±1.24 <sup>a</sup>
Group II (Toxin treatment)				
100 mM ethanol	30.26±1.42 <sup>a</sup>	235.6±6.22 <sup>a</sup>	34.38±2.15 <sup>a</sup>	44.36±2.84 <sup>a</sup>
Group III Silymarin treatment				
100 mM ethanol + Silymarin(50 µg/mL)	76.31±1.81 <sup>a</sup>	156.31±3.16 <sup>a</sup>	15.17±2.14 <sup>a</sup>	20.14±2.17 <sup>a</sup>
100 mM ethanol + Silymarin(100 µg/mL)	84.72±1.35 <sup>a</sup>	144.23±4.32 <sup>a</sup>	13.31±1.19 <sup>a</sup>	18.36±1.33 <sup>a</sup>
Group IV (LCME treatment)				
100 mM ethanol + LCME (50 µg/mL)	55.15±1.01 <sup>a</sup>	199.3±1.26 <sup>a</sup>	31.61±2.35 <sup>a</sup>	34.31±1.27 <sup>a</sup>
100 mM ethanol + LCME (100 µg/mL)	71.16±1.52 <sup>a</sup>	175.21±3.14 <sup>a</sup>	22.64±2.31 <sup>a</sup>	24.34±2.05 <sup>a</sup>
100 mM ethanol + LCME (200 µg/mL)	63.33±2.03 <sup>a</sup>	189.24±4.43 <sup>a</sup>	24.4±2.18 <sup>a</sup>	29.36±2.67 <sup>a</sup>

Data represent mean ± S.D (n=6). *p* value-Normal Vs other groups; *p* <0.01<sup>a</sup>, *p* <0.05<sup>b</sup>.

The results of the study are shown in Table 1. Ethanol is metabolized by alcohol dehydrogenase in liver. But in conditions like alcohol abuse, the microsomal electron transport system also participates in ethanol oxidation via cytochrome P 450 (2E1, 1A2 and 3A4 isoforms) isoenzyme, which generates free radicals causing cell damage<sup>10</sup>. Treatment with ethanol (100mM) alone caused a drastic decrease in cell viability i.e., 31.27±1.02% viability. Inoculation of ethanol increased the levels of AST, ALT and LDH indicating damage to the cell plasma membrane as these enzymes are normally present in cytoplasm<sup>11</sup>. Treatment with LCME at 50, 100 and 200 µg/mL significantly reduced the elevated level of these enzymes in ethanol treated groups indicating the hepatoprotective activity of the extract. The percentage cell viability as well as the reduction in the elevated enzyme levels in ethanol treated groups was maximum with LCME at 100µg/mL. The significant reversal effect of the extract on the change in the altered level of these enzymes may be attributed to the antioxidant principles present in the extract such as flavonoidal and phenolic compounds, which may enable the extract in preventing the leakage of these enzymes into the supernatant by preserving the structural integrity of hepatocellular membrane.

Methanolic extract of *Lindernia ciliata*(LCME)

## CONCLUSION

The protective effect of extract may be due to its ability to scavenge free radicals that are produced during the metabolism of ethanol in HepG2 cells. The antioxidant principles such as flavonoids and phenolic compounds present in the extract, LCME contribute to the hepatoprotective activity of the extract. Further, the findings of the study substantiate the usage of the plant in traditional medicine for the treatment of jaundice.

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## CONFLICT OF INTEREST STATEMENT

All the authors declare that there is no conflict of interest.

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