**In vitro Hepatoprotective Activity of Barleria montana Nees Leaves against Anti-Tubercular Drugs Induced Hepatotoxicity in BRL3A Cell Lines**

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**ABSTRACT**

*Barleria Montana* Nees traditionally used for treating wounds, diabetes, cuts, inflammation and hepatoprotective activity. The study was designed to evaluate the hepatoprotective activity of ethanolic extract of *Barleria Montana* Nees (BMEE) leaves against anti-tubercular drugs induced hepatotoxicity in BRL3A cell lines using MTT assay. The cytotoxicity (CTC50) of anti-tubercular drugs on BRL3A cell lines was evaluated by MTT assay. The CTC50 of BMEE was tested and the percentage viability of the cell lines was carried out in triplicates. Silymarin was used as a standard drug to compare with anti-tubercular drugs and BMEE. Finally, the estimation of liver biomarker and antioxidant enzymes like AST, ALT, LDH, GSH and MDA in BRL3A cell lines homogenate. The CTC50 for anti-tubercular drugs was obtained approximately 3800μg/ml. This concentration of 3800μg/ml was used to induce hepatotoxicity and to determine the hepatoprotective effect of the BMEE on BRL3A cell lines using MTT assay. The CTC50 of BMEE was found to be 739.33μg/ml. Elevated levels of AST, ALT, LDH, GSH and decreased MDA in BRL3A cell lines homogenate after anti-TB drugs administration. The pre-treatment of BMEE with anti-TB drugs were significantly reversed these enzymes in BRL3A cell lines homogenate. The present study was explored that the ethanolic extract of *Barleria Montana* Nees leaves have protective effect on hepatotoxicity induced by anti-TB drugs in BRL3A cell lines.

**Keywords:** Cytotoxicity (CTC50), BMEE, Anti-TB drugs, MTT assay, BRL3A cell lines.

**INTRODUCTION**

Hepatotoxicity is a liver disorder caused by a wide variety of pharmaceutical agents, natural products, chemicals or environmental pollutants and dietary constituents. Some of the commonly used drugs leading to hepatotoxicity are non-steroidal anti-inflammatory agents,¹ first line anti-tubercular drugs,² ³ ⁴ anti-viral drugs,⁵ anti-epileptic drugs,⁶ anti-malarial drugs,⁷ anti-hyperlipidaemics⁸ and commonly abused alcoholic beverages. In patients using the anti-tubercular drugs, oxidative stress is the important mechanism in causing the hepatotoxicity. Isoniazid causes increase in the liver aminotransferases and clinical hepatitis leading to the nausea, vomiting and loss of appetite. Rifampicin may cause hepatitis and jaundice and Pyrazinamide known to cause the hepatotoxicity.⁹

Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse drug reactions further damaging the liver. In modern medicine, there are not much drugs available for the treatment of liver diseases. Management of liver diseases is still a challenge to modern medicine. Hence, it is mandatory to search for alternative treatment-supplementation for the treatment of liver disease in order to replace currently used conventional drugs.

*Barleria montana* Nees commonly known as Mountain Barleria, is an erect herb found in the mountains of Western Ghats. It is native to a wide area ranging from southern China to India and Myanmar. It is traditionally used for treating wounds, diabetes, cuts, inflammation and hepatoprotective activity.¹⁰ The chemical constituents of *Barleria montana* Nees are coumarin, sterol, quinine, flavonoids, alkaloids, terpenoids and tannins. Twenty-six phytoconstituents were found by GC-MS analysis of this plant.¹¹

The aim of the present study was supplementation of plant drug ethanolic extract of *Barleria Montana* Nees (BMEE) along with anti-tubercular drugs used to reduce the risk of drug induced hepatotoxicity.

**MATERIALS AND METHODS**

**Preparation of Plant material**

The leaves of *Barleria Montana* Nees were collected from tirumala hills, A.P. Plant was identified, authenticated and certified (Voucher no: 32) by Dr.K.Madhavachetty, Asst.Professor, Dept. of Botany, S.V.University, Tirupati, A.P, India. The air-dried leaves powder was extracted in soxhlet apparatus using ethanol as solvent. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extract was concentrated to 3/4th of the original volume by distillation.
Chemicals
3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco’s Modified Eagle’s Medium (DMEM) and trypsin were purchased from Sigma Aldrich Co, St Louis, USA. Tris base and antibiotics from Hi-Media Laboratories Ltd., Mumbai; Dimethyl Sulfoxide (DMSO), ethanol and Propanol from E. Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium
BRL3A (a rat, liver derived cell line) culture cell was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of BRL3A were cultured in DMEM supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5μg/ml) in a humidified atmosphere of 35% CO2 at 37°C until confluent. The cells were dispersed with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Reagents Preparation
BRL3A cell lines – A rat liver derived cell lines originally isolated by primary clone.

Anti-Tubercular Drugs
Ethambutol, Cap R Cinex (Isoniazid and rifampicin) and Pyrazinamide were mixed in 2:1:5 proportions to make hepatotoxicant solution 2 (HTS2) and tested in different concentrations.

Preparation of Test Solutions
The test drugs were separately weighed and dissolved in distilled DMSO and volume was made to 1mg/ml concentration of stock solution by DMEM supplemented with 2% inactivated FBS and sterilized by filtration. Two Serial fold dilutions were prepared from the above stock solution and this is used for carrying out cytotoxic studies.

Determination Cytotoxicity (CTC50) for Anti-Tubercular Drugs and BMEE in BRL3A Cell Line by MTT Assay

The monolayer cell culture was trypsinized and cell suspension was prepared from which the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. 50μl of DMEM with nontoxic concentration (3800μg/ml final volume) of toxicant and 50μl of different non-toxic test concentrations of plant extracts and silymarin were added separately to the cell suspension.

Then, the plates were incubated at 37°C for 24h in 5% CO2 atmosphere. After 24h, the cell supernatants were discarded and 50μl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3h at 37°C in 5% CO2 atmosphere. The supernatant was removed and 100μl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm.

Calculations: The percentage growth inhibition was calculated using the following formula and concentration of test drugs needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

\[ \% \text{ of Growth inhibition} = \left( \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right) \times 100 \]

Determination of Hepatoprotective Activity of Plant Extracts and Silymarin

The monolayer cell culture was trypsinized and the cell suspension was prepared form which the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. 50μl of DMEM with nontoxic concentration (3800μg/ml final volume) of toxicant and 50μl of different non-toxic test concentrations of plant extracts and silymarin were added separately to the cell suspension.

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The percentage cell viability was determined, based on which the percentage protection offered by plant extracts and silymarin was calculated over the toxicant control. All the tests were done in triplicates. The treatment schedule as follows Group 1: Normal treated with media (50 μl/ml) as vehicle control, Group 2: Positive control treated with HTS2 (3800μg/ml), Group 3: Standard control treated with silymarin (200μg/ml) and HTS2, Group 4: Test 1 treated with BMEE (25μg/ml) and HTS2; and Group 5: Test 2 treated with BMEE (50μg/ml) and HTS2. Finally, the BRL3A cell lines were processed to homogenization used to estimate the liver biomarker enzymes and antioxidant enzymes.
Determination of Biochemical and Antioxidant Parameters in BRL3A Cell Lines Homogenate

Aspartate Aminotransferase (AST)\textsuperscript{15}

**Procedure**

1ml of buffered substrate was incubated at 37°C for 10 minutes. Then added 0.2ml of cell culture medium to the test tubes and again incubated at 37°C for 30 minutes. The reaction was arrested by adding 1.0ml of DNPH reagent and tubes were kept at room temperature for 20 minutes. Then 10ml of 0.4N sodium hydroxide solution was added. A set of pyruvic acid was also treated in a similar manner for the standard. The colour developed was read at 520nm. The activity was expressed as IU/L.

Alanine aminotransferase (ALT)\textsuperscript{15}

**Procedure**

1ml of buffered substrate was incubated at 37°C for 10 minutes. Then added 0.2ml of cell culture to the test tubes and again incubated at 37°C for 30 minutes. The reaction was arrested by adding 1.0ml of DNPH reagent and the tubes were kept at room temperature for 20 minutes. Then 10ml of 0.4N sodium hydroxide solution was added. A set of pyruvic acid was also treated in a similar manner for the standard. The colour developed was read at 520nm. The activity was expressed as IU/L.

Lactate dehydrogenase (LDH)\textsuperscript{16}

**Procedure**

To 0.1ml of the buffered substrate, 0.1ml of cell culture medium was added and the tubes were incubated at 37.8°C for 15 minutes. The incubation was continued for another 15 minutes after adding 0.2ml of NAD+ solution. The reaction was arrested by adding 0.1ml of 2,4-DNPH, and the tubes were incubated for a further period of 15 min at 37.8°C after which 7.0ml of 0.4N sodium hydroxide was added and the colour developed was measured at 420nm. The activity was expressed as IU/L.

Reduced Glutathione Level (GSH)\textsuperscript{17}

**Procedure**

Take 0.1ml of BRL3A cell lines homogenate and precipitated with 5% TCA (Trichloroacetic Acid). The contents were mixed well for complete precipitation of protein and centrifuged. To 0.1ml of supernatant, added 2ml of 0.6Mm DTNB [5, 5 dithiob (2-nitrobenzoic acid)] reagent and this volume made up to 4ml by added 0.2M phosphate buffer (pH 8). Then the absorbance was read 412nm. The amount of glutathione was expressed as n moles / mg of protein.

Lipid peroxidation (MDA Level)\textsuperscript{18}

**Procedure**

To 0.2ml of BRL3A cell lines homogenate, 0.2ml of 8.1% SDS, 1.5ml of 20% acetic acid (pH 3.5) and 1.5ml of 0.8% TBA were added. The above solution was made to 4ml with water and then heated in a water bath at 95.80°C for 60min using glass ball as a condenser. After cooling, 1ml of water and 5ml of n-butanol: pyridine (15:1 v/v) mixture were added and shaken vigorously. Then the solution was centrifuged at 4000rpm for 10 min, in which the organic layer was taken and measured their absorbance at 532nm. The level of lipid peroxides was expressed as ‘n’ moles of MDA/ mg of protein.

Statistical analysis

The results were presented as Mean ± SEM. Statistical significance between the normal and control, control and treatment groups were observed using One-way ANOVA followed by Tukey Post Hoc method.

**RESULTS**

Cytotoxicity (CTC50)

CTC50 was determined for the anti-tubercular drugs by MTT assay. Dose response curve was drawn for the different concentrations of anti-tubercular drugs and CTC50 was obtained approximately 3800μg/ml was shown in Figure 1. This concentration (3800μg/ml) was used to induce hepatotoxicity and to determine the hepatoprotective effect of the plant extract on BRL3A cell lines using MTT assay. The CTC50 of BMEE was also found to be 739.33μg/ml which can be observed from figure 2. Silymarin at a dose of 200μg/ml was showed highest protection (60.09%) in BRL3A cell lines over positive control. The selected plant extract for the study BMEE was showed protection 36.41% at a dose of 50μg/ml and 14.27% at a dose of 25μg/ml in BRL3A cell lines shown in Figure 3.

Effect of BMEE on AST, ALT and LDH in BRL3A cell lines homogenate

In the present study, biochemical parameters like AST, ALT and LDH were assessed in the BRL3A cell lines suspension. Administration of HTS2 to the BRL3A cell lines had shown significantly (P<0.001) increased AST, ALT and LDH levels in BRL3A cell lines when compared with HTS2 alone treated suspension. Administration of BMEE 25μg/ml along with HTS2 had shown significantly (P<0.001) decreased AST, ALT and LDH levels in BRL3A cell suspension when compared with the positive control which was given in Table 1.

Co-treatment BMEE 25μg/ml along with HTS2 had shown significantly (P<0.001) decreased AST, ALT and LDH levels in BRL3A cell suspension when compared with positive control. BMEE 50μg/ml treated group along with anti-tubercular drugs has showed significantly (P<0.001) decreased AST, ALT and LDH levels in BRL3A cell suspension when compared with HTS2 alone treated groups.

Effect of BMEE on Malondialdehyde and Reduced Glutathione in BRL3A Cell Lines Homogenate

Administration of HTS2 to the BRL3A cell lines had significantly (P<0.001) increased malondialdehyde and reduced glutathione levels when compared with control.
group. Silymarin 200μg/ml treated cell lines was showed significantly (P<0.001) decreased malondialdehyde and increased glutathione levels when compared with positive control group reported in Table 2. Treatment of BMEE 25μg/ml with HTS2 had shown significantly (P<0.001) decreased in malondialdehyde and increased in glutathione levels in BRL3A cell suspension when compared with the positive control group. BMEE 50μg/ml treated with HTS2 (anti-tubercular drugs) was showed significantly (P<0.001) decreased malondialdehyde and increased glutathione levels in hepatocytes homogenate when compared with the positive control group.

![Figure 1: Cytotoxicity for Anti-Tubercular Drugs in BRL3A cell lines by MTT assay](image1)

![Figure 2: CTC50 of BMEE in BRL3A cell lines by MTT Assay](image2)

![Figure 3: Hepatoprotective Activity of Plant Extract (BMEE) & Silymarin](image3)
The principle involved in MTT assay was involves 3-(4, 5 dimethylthiazole-2-yl)-2, 5 diphenyl tetrazolium bromide) is taken up into the cells and reduced to a blue color by mitochondrial dehydrogenases. The formazan product accumulates within the cell, due to the fact that it cannot pass through plasma membrane. On solubilization of the cells, the product is liberated and can be readily detected and quantified by a simple colorimetric method.

The ability of the cells to reduce MTT provides an indication of mitochondrial integrity and activity which in turn may be interpreted as a measure of viability and/or cell number. The assay has been adopted for use with cultures of exponentially growing cells. Determination of their ability to reduce MTT to the formazan derivative after exposure to test compounds compared to control, enables the relative protection of test chemicals to be assessed.  

The present study involves, determined CTC50 of the anti-tubercular drugs by MTT assay. Dose response curve was drawn for the anti-tubercular drugs in different concentrations.  

Table 1: In Vitro Hepatoprotective Activity of BMEE on Biochemical Parameters in BRL3A Cell Lines

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal</td>
<td>18.37 ± 0.2691</td>
<td>21.68 ± 0.3051</td>
<td>126.27 ± 0.6871</td>
</tr>
<tr>
<td>Group II</td>
<td>Positive control (HTS₂)</td>
<td>63.177 ± 1.276***</td>
<td>73.227 ± 2.836***</td>
<td>261.47 ± 4.102***</td>
</tr>
<tr>
<td>Group III</td>
<td>Silymarin (200μg/ml) + HTS₂</td>
<td>24.413 ± 0.6454***</td>
<td>29.09 ± 0.335***</td>
<td>132.22 ± 1.728***</td>
</tr>
<tr>
<td>Group IV</td>
<td>BMEE (25μg/ml) + HTS₂</td>
<td>35.55 ± 1.126***</td>
<td>45.077 ± 1.192***</td>
<td>148.78 ± 3.364***</td>
</tr>
<tr>
<td>Group V</td>
<td>BMEE (50μg/ml) + HTS₂</td>
<td>29.09 ± 0.132***</td>
<td>34.743 ± 0.4286***</td>
<td>133.67 ± 3.949***</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E.M (n=6), One-way ANOVA Tukey post Hoc; ***p≤0.001 vs. Normal (Group I); **p≤0.001 vs. Positive control (Group II).

Table 2: In Vitro Hepatoprotective Activity of BMEE on Antioxidant Parameters in BRL3A Cell Lines Suspension

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>MDA (n mol/mg protein)</th>
<th>GSH (n mol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>9.647 ± 0.179</td>
<td>35.953 ± 0.2206</td>
</tr>
<tr>
<td>Group II</td>
<td>Positive control (HTS₂)</td>
<td>35.390 ± 1.284***</td>
<td>11.830 ± 1.997***</td>
</tr>
<tr>
<td>Group III</td>
<td>Silymarin (200μg/ml) + HTS₂</td>
<td>12.330 ± 0.225***</td>
<td>27.783 ± 1.312***</td>
</tr>
<tr>
<td>Group IV</td>
<td>BMEE (25μg/ml) + HTS₂</td>
<td>20.793 ± 0.3206***</td>
<td>20.76 ± 0.338***</td>
</tr>
<tr>
<td>Group V</td>
<td>BMEE (50μg/ml) + HTS₂</td>
<td>15.530 ± 0.2524***</td>
<td>25.817 ± 0.1855***</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E.M (n=6), One-way ANOVA Tukey post Hoc; ***p≤0.001 vs. Normal (Group I); **p≤0.001 vs. Positive control (Group II).

DISCUSSION

Liver is considered as the most important vital organ in performing the xenobiotic metabolism, detoxifying the drugs and eliminating them from the body providing protection against foreign substances. Liver cell injury is caused by the various toxic substances like antibiotics, chemotherapeutic agents, CCl4, Excessive alcohol consumption and Microbes etc. Despite tremendous advances made in the pharmaceutical field no significant hepatoprotective agents are effective against hepatic disorders. Therefore, due importance was given to develop plant based hepatoprotective agents.

The present study was aimed at compiling data based report on plant based phytochemicals tested on anti-tubercular drugs induced hepatotoxicity models using cell lines.

The in vitro hepatoprotective activity was assessed in BRL3A cell lines (a rat liver-derived cell lines) against anti-tubercular drugs induced hepatotoxicity. The antitubercular drugs (Ethambutol, Cap R Cinex (isoniazid and Rifampicin) and Pyrazinamide) were mixed in 2:1:5 proportions to make HTS2 solution used to induce hepatotoxicity. In this, first determined CTC50 (cytotoxicity) for anti-tubercular by MTT assay and then estimated liver biomarker enzymes in BRL3A cell suspension for the protective effect of BMEE on hepatocytes.

The principle involved in MTT assay was involves 3-(4, 5 dimethylthiazole-2-yl)-2, 5 diphenyl tetrazolium bromide) is taken up into the cells and reduced to a blue colour formazan product in a mitochondrial dependent reaction. The formazan product accumulates within the cell, due to the fact that it cannot pass through plasma membrane. On solubilization of the cells, the product is liberated and can be readily detected and quantified by a simple colorimetric method.

The ability of the cells to reduce MTT provides an indication of mitochondrial integrity and activity which in turn may be interpreted as a measure of viability and/or cell number. The assay has been adopted for use with cultures of exponentially growing cells. Determination of their ability to reduce MTT to the formazan derivative after exposure to test compounds compared to control, enables the relative protection of test chemicals to be assessed.  

The present study involves, determined CTC50 of the anti-tubercular drugs by MTT assay. Dose response curve was drawn for the anti-tubercular drugs in different concentrations.
concentrations like 4000μg/ml (56.44±0.3), 2000μg/ml (25.61±0.4), 1000μg/ml (19.37±0.3), 500μg/ml (17.10±0.5), 250μg/ml (10.38±0.1) and 125μg/ml (8.78±0.3).

From this dose response curve, the CTC50 of anti-tubercular drugs was calculated i.e. concentrations of hepatotoxicant required to reduce cell viability. The concentration of hepatotoxicant was used to induce toxicity in BRL3A cell lines and evaluated the protective effect of BMEE. Since approximately 50% of the inhibition on BRL3A cell lines was achieved with 3800μg/ml. So, CTC50 of anti-tubercular drugs was taken as 3800μg/ml to induce hepatotoxicity. The CTC50 concentration for BMEE was also calculated by MTT assay i.e. 739.33μg/ml. From these two graded concentrations of 25μg/ml and 50μg/ml were chosen to investigate the hepatoprotective activity of BMEE.

From results of the in vitro hepatoprotective study in BRL3A cell lines, anti-tubercular drugs (HTS2) were showed significant reduction in the protection of hepatocytes and also reduction in the percentage viability of cells. In BMEE treatment along with HTS2 showed protection on BRL3A cell lines i.e. 25μg/ml (14.27±3.36) and 50μg/ml (36.41±5.94). Silymarin 200μg/ml (60.09±4.06) has also showed protection on BRL3A cell lines over HTS2 (anti-tubercular drugs) induced toxicity.

The AST and ALT are well known diagnostic markers of liver diseases. Lactate dehydrogenase (LDH) is an enzyme present in the cytoplasm of hepatocytes, catalyzes the interconversion of lactate and pyruvate. Exposure of anti-tubercular drugs (HTS2) to BRL3A cell lines an abnormal raise in the levels of AST, ALT and LDH. In case of liver damage with hepatocellular lesions and parenchymal cell necrosis, these liver marker enzymes are released from the damaged tissues into the blood stream in conformity with the extent of liver damage.19 Treatment with BMEE 25μg/ml and 50μg/ml significantly reduced the elevation in liver enzymes. Hence, BMEE showed protection and it may be involved in the stabilization of hepatocytes plasma membrane, from the leakage of cytoplasmic enzymes AST, ALT and LDH.

Anti-tubercular drugs produced a significant depletion of protective peptide GSH which is an important non-enzymatic antioxidant that plays a crucial role in scavenging ROS and maintaining enzymatic antioxidants. Administration of anti-tubercular drugs (HTS2) elevates the formation of lipid peroxides and ROS, leading to the inactivation of enzymatic and non-enzymatic antioxidants in the liver.20 Depletion of GSH obtained by anti-tubercular drugs exposure may be attributed to its rapid utilization by the overproduction of ROS and subsequent oxidative stress. From the results of the study, hepatic GSH levels were increased by the treatment of BMEE 25μg/ml and 50μg/ml. Hence, it indicates that detoxification of ROS leads to the prevention of hepatotoxicity.

The hepatic MDA, a major reactive aldehyde resulting from the peroxidation of polyunsaturated fatty acids in the cell membrane, reflects a causal role of lipid peroxidation.21 In anti-tubercular drugs (HTS2) treatment elevated the levels of hepatic MDA causes liver damage. By the treatment of BMEE 25μg/ml and 50μg/ml showed a significant inhibitory effect on lipid peroxidation and thereby reduced the anti-tubercular drugs induced hepatic membrane destruction and hepatic damage.

Treatment with BMEE significantly reversed all these abnormal changes in hepatocytes and thus offered hepatoprotection against anti-tubercular drugs induced hepatotoxicity in BRL3A cell suspension.

CONCLUSION

Drug induced liver injury is a major issue for the pharmaceutical companies for the failure of the drug to get approved or for withdrawal of already marketed product. Anti-TB drug induced hepatotoxicity causes substantial morbidity and mortality diminishing the treatment effectiveness. Many phytochemical constituents were detected in the plant parts that have beneficial importance in medical sciences. In the current study, the CTC50 value for anti-tubercular drugs was calculated by MTT assay using BRL3A cell lines. CTC50 of anti-tubercular drugs 3800μg/ml was used for the in vitro hepatoprotective activity of anetholic extract of *Barleria Montana* Nees. From the results of the present study, it was confirmed that anetholic extract of *Barleria Montana* Nees exhibited in vitro hepatoprotection in BRL3A cell lines. Thereby the plant extract can be used as supplementation/ adjuvant with anti-tubercular drugs to reduce the ADR of hepatotoxicity caused by anti-tubercular drugs.

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