Evaluation of Hepatoprotective action of *Andrographis paniculata* in *in-vitro* cultured Hepatocytes

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**A R T I C L E  I N F O**

**ABSTRACT**

Liver plays a vital role in metabolism and detoxification. Impairment due to injury or infection leads to deterioration of function may imparts many implications on one’s health. Till date treatment for liver diseases by modern medicine is a challenge. Only phytocconstituents are known remedies for the liver management while allopathic medicine has little to offer for the hepatic ailments. *Andrographis paniculata* is well known plant in Bengal by the name ‘kalmegh’ Kalamegha, meaning “dark cloud”. In present study single cell suspension of hepatocytes was isolated from adult wister mice through mechanical shearing, and cultured in culture petriplates in RPMI1640 with 2% agarose to provide 2D frame, supplemented with 5% FBS and streptomycin (100µg ml⁻¹). Aqueous extract and alcoholic extract of *Andrographis paniculata* were selected, Liv52 was selected as control drug. Hepatotoxicity was induced by 15mM CCl₄. Morphological investigation of cultured cells and microculture tetrazolium assay (MTA Assay), confirms that hepatoprotective ability at higher dose. Dose dependent increase in viability was recorded in hepatocytes exposed to CCl₄. These finding prove the efficiency of hepatoprotective activity of *Andrographis paniculata* in *in vitro* culture by normalizing biochemical actions altered by CCl₄ intoxication.

**Introduction**

*Andrographis paniculata*, commonly known as Siriyangai in Tamil, belongs to the family Acanthaceae is widely used in the Indian traditional system of medicine. The major component of *A. paniculata* is andrographolide is a bitter, colorless, and crystalline in appearance, is called diterpene lactone (Siripong et al., 1992). Recent research has revealed that *Andrographis paniculata* has a surprisingly broad category of pharmacological activity and some of them are enormously beneficial, such as anti-inflammatory, antiarrhoeal, antiviral, antimalarial (Shen et al., 2002), hepatoprotective, cardiovascular, anticancer, and immune-stimulatory activities (Calabrese et al., 2000). *Andrographis paniculata* is used to treat poisonous bites, diabetes and respiratory tract infection. The plant possesses anti-inflammatory, antipyretic, antiviral, immune stimulatory, anticancer, anti-hyperglycemic and antioxidant properties (Vijayakumar et al., 2007). Many of the plants are rich in secondary metabolites and are potent source of drugs. On the other hand, male reproductive toxicity and cytotoxicity of the plant Andrographis has also been reported.

**Materials and Methods**

Plant extract and drug selected for study

*Andrographis paniculata* was selected for the present study. Aqueous extract and alcoholic extract were prepared by adopting methods (Trease and Evans, 1978, Kokatte, 2006) and selected as a test samples, Liv52 (Himalaya product) was selected as control drug. Hepatotoxicity was induced by 15mM CCl₄. Morphological investigation of cultured cells and micro culture tetrazolium assay (MTA Assay), confirms that hepatoprotective ability at higher dose. Dose dependent increase in viability was recorded in hepatocytes exposed to CCl₄. These finding prove the efficiency of hepatoprotective activity of *Andrographis paniculata* in *in vitro* culture by normalizing biochemical actions altered by CCl₄ intoxication.

**Stock solution preparation**

For hepatoprotective and hepatotoxicity activity in *in vitro* studies, stock solution of extract and drug at various dilutions were prepared with DMSO for better uptake of molecule and non toxic property.

**Cell preparation**

In present study single cell suspension of hepatocytes was prepared from liver of adult Wister mice through mechanical shearing (Ursal et al., 2014), and cultured in culture petriplates in RPMI1640 with 2% agarose to provide 2D frame, supplemented with 5% FBS and streptomycin (100µg) in CO₂ incubator with 5% CO₂ at 35°C or 24 hrs, then cells were subjected to CCl₄ treatment for toxicity.
### Table-1: Protective effect of given extracts on CCl₄ induced toxicity on cultured liver cells

<table>
<thead>
<tr>
<th>Cells and treatment</th>
<th>Conc. in (µg ml⁻¹)</th>
<th>% viability of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>800</td>
<td>65.75±1.94</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>250</td>
<td>75.73±1.84</td>
</tr>
<tr>
<td>Liv52</td>
<td>230</td>
<td>78.83±1.94</td>
</tr>
<tr>
<td>CCl₄</td>
<td>15mM</td>
<td>32.21±1.02</td>
</tr>
</tbody>
</table>

**Figure-1: Hepatocytes treated with Liv52**

**Figure-2: Hepatocytes treated with alcoholic extract of *Andrographis paniculata***

**Figure-3: Hepatocytes treated with aqueous extract of *Andrographis paniculata***
Determination of Mitochondrial Synthesis by Microculture Tetrazolium (MTA Assay).
The ability of the cells to survive a toxic treatment has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay requires both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTA to a blue formazan derivative by living cells in clearly a very effective principle on which the assay is based (Freshney, 2000).

In vitro hepatoprotective activity against CCl₄ induced toxicity
Below the CTC₅₀ value two dose levels were selected for each extract and used for further studies. In present study modified method of Vijayan et al., 2002 monolayer was washed once and treated with 100µl of different drug concentrations for 24 hrs. After 24 hrs of pretreatment with the extracts, the cells were challenged with CCl₄ (15 mM) where 100µl of different drug concentration and 100µl of CCl₄ was added. The plates were then incubated at 37°C for further 24 hours in 5% CO₂ atmosphere. Microscopic examination was carried out and observations were recorded every 24 hrs. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTA in RPMI 1640 was added to each well. The supernatant was removed and 50µl of propanol was added to solubilize the formed formazan.

Morphological analysis
Cells were examined microscopically for any morphological change induced by treatment of drug and CCl₄.

Results and Discussion
Liver injuries induced by CCl₄ are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and or hepatoprotective activities of drugs (Clawson 1989, Lin et al., 2002). Percentage of viable cells exposed to extract CCl₄ and Liv52 were given in table1. It was suggested that, CCl₄ get accumulated and metabolically activated in hepatic parenchyma cells which is cytochrome P450 dependent monooxygenases to form trichloromethyl radical(CCl₃). The CCl₄ radical acts on cellular proteins to alkylates and other macromolecules simultaneous attack on polysaturated fatty acids, in the presence of oxygen to produce lipid peroxides, resulted in liver damage. Thus it was suggested by Bishayee et al., 1995, that antioxidant or free radical generation inhibition is important in protection against CCl₄ induced liver damage. Which was supported by Muthu et al., (2008) CCl₄ induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST).

Figure-4: Hepatocytes treated with CCl₄ large no of nonviable cells due to stress

References


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