EVAlUATION OF THE CYTOTOXIC ACTIVITY OF CRUDE AQUEOUS EXTRACTS OF SOME MEDICINAL PLANTS OF KASHMIR

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Abstract: The objective of this study was to evaluate the cytotoxicity potential of aqueous extracts of Nepeta cataria, Fumaria indica, Borago officinalis, Adiantum capillus, Levandula stoeches on HeLa cell line. Leaves of plants were washed with distilled water, dried in shade, grinded to fine powder and stored in airtight container at room temperature in the dark until used. The powdered samples were subjected to extraction using distilled water. Different concentrations of the extracts of herblas were prepared. Cytotoxicity study was carried out by The MTT cell proliferation assay on HeLa cell line which measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, there is reduction in cell viability. Fumaria indica exhibited cell viability ranging from 89.60 to 100 per cent against the extract concentration of 5 to 100 μg/ml with IC_{50} (µg/ml) on HeLA cell line as -581.79 Adiantum capillus extract revealed cell viability from 81.91 to 100 per cent against the extract concentration of 5 to 100 μg/ml with IC_{50} (µg/ml) on HeLA cell line as 36.96. Nepata cataria extract showed cell viability ranging from 84.36 to 96.09 per cent against the extract concentration of 5 to 100 μg/ml with IC_{50} (µg/ml) on HeLA cell line as -112.07. In case of Levandula stoeches extract per cent cell toxicity ranged from 0 to 41.77 against the extract concentration of 5 to100 μg/ml. Borago officinalis extract exhibited per cent cell toxicity ranging from 4.27 to 43.17 against the extract concentration of 5 to100 μg/ml with IC_{50} (µg/ml) on HeLa cell line as 26.91.

Key words: Medicinal plants, Cytotoxicity

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. A wide range of medicinal plant parts are used for extract of raw drugs and they possess varied medicinal properties. WHO has recommended to all member countries to actively promote native medicines of their respective country and initiate steps to conserve medicinal plants and integrating traditional health care facilities [2]. The use of conventional plant products described in ancient literature in modern medicine suffers from the fact that scientific evidence and explanation are lacking in most cases. These practices are potentially toxic or harmful. Indeed, it is difficult to measure these systemic effects in vivo. Considering the vast potentiality of plants as sources for antimicrobial and anti-inflammatory drugs, the present study was undertaken to screen the cytotoxic potential of crude aqueous extracts of some plants. It is important for the purpose of determining the potential toxicity of the compounds being studied. Cytotoxicity studies of local plant extracts or folk medicinal plant extracts has not been studied extensively and this is vital for the safety evaluation of any herbal
preparations. Thus the objective of this study was to evaluate the potential cytotoxicity of aqueous extracts of 5 medicinal plants viz., Nepeta cataria, Fumaria indica, Borago officinalis, Adiantum capillus, Levandula stoeches using HeLa cell line.

**MATERIAL METHODS**

**Plant material:** Crude aqueous extract of leaves of Nepeta cataria, Levandula stoeches, Fumaria indica, Adiantum capillus and Borago officinalis were evaluated for their in-vitro cyto-toxicity trial on cell line (HeLa cell lines).

The selected herbs (leaves) were purchased from registered herbal shops from local market Srinagar, J&K. Plant material was washed with distilled water, dried in shade, grinded to fine powder and stored in airtight container at room temperature in the dark until used. The powdered samples were subjected to extraction using distilled water following the method of Nair et al. [3]. Different concentrations of the extracts of herbs were prepared.

**In vitro toxicity study on cell line:** Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population’s response to external factors. The MTT cell proliferation assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, there is reduction in cell viability.

DMEM (Dulbecco’s modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA phosphate buffered saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and fetal bovine serum (FBS) were purchased from Gibco. 25 cm² and 75 cm² flask and 96 well plated purchased from eppendorf India.

**Maintenance of cell line:** The Hela cervical cancer cell line were purchased from NCCS, Pune and the cells were maintained in DMEM supplemented with 10% FBS and the antibiotics penicillin streptomycin (0.5 mL⁻¹), in atmosphere of 5% CO₂/95% air at 37°C.

**Preparation of test compound:** For MTT assay, each test compounds were weighed separately and dissolved in DMSO. With media make up the final concentration to 1 mg/ml and the cells were treated with series of concentrations from 10 to 100 µg/ml.

**HeLa cell viability by MTT assay:** MTT assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured formazan crystals. The cells are then solubilized with DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

**Procedure:** Cell viability was evaluated by the MTT assay with three independent experiments with six concentrations of compounds in triplicates. Hela cells were trypsinized and perform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 × 103 cells/well in 100 µl media in 96 well plate culture medium and incubated overnight at 37°C. After incubation, remove the old media and add fresh media 100µl with different concentrations of test compound in representative wells in 96 plate. After 48 hrs discard the drug solution and add the fresh medic with MTT solution (0.5 mg/mL⁻¹) was added to each well and plates were incubated at 37°C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% values is generated from the dose-response curves for each cell line using with origin software.

\[
\text{Per cent inhibition} = \frac{100 \times (\text{control}-\text{treatment})}{\text{Control}}
\]

**RESULT AND DISCUSSION**

The results of study are depicted in table 1. It is evident that Adiantum capillus cell viability is
ranged from 81.91 to 100 per cent against the extract concentration of 5 to 100 μg/ml with IC50 (µg/ml) 36.96 on HeLa cell line. Gaikwad et al. [4] also reported that the histological damages in Adiantum capillus extract treated rats with the dose rate of 500mg/kg b.wt. for 14 days were minimal in contrast with the toxic drug cisplatin on rat. Ethanolic extract Adiantum capillus veneris showed good antioxidant activity and it exhibited low IC50 value (0.3986 mg/gm) for DPPH assay and 0.695 mg/gm for ABTS assay and results obtained indicated that leaves of this plant are endowed with free radical scavenging molecules and it can be used as a potential source of natural antioxidants and nutrients [5]. Paul et al. [6] revealed that 2000 mg/kg of Adiantum Philippense on mice for 14 days was safe and concluded that 500mg and 250mg/kg b.wt of Adiantum philippense was chosen for subsequent experimentation.

Fumaria indica exhibited cell viability from 89.60 to 100 per cent against the extract concentration of 5 to 100 μg/ml with IC50 (µg/ml) on HeLa cell line as - 581.79, while as per cent cell toxicity of Fumaria indica ranged from 0 to 12.53 against the extract concentration of 5 to 100 μg/ml. The leaves extract of plant was found to be safe in cytotoxic test and devoid of toxic manifestations during chronic administration [7]. Erdagon [8] also reported that the water extract of Fumaria indica showed no cytotoxicity activity.

Borago officinalis extract exhibited cell viability which ranged from 47.24 to 71.6 per cent against the extract concentration of 5 to 100 μg/ml with IC50 (µg/ml) on HeLa cell line as 26.91. This is in contrast to the study of Nawaz et al. [9], who evaluated acute and repeated dose toxicity of the Polyherbal Formulation Linkus Syrup containing Borago officinalis as one of its components in rats. At doses of 20, 500 and 1000 mg/kg did not cause any sign of toxicity like hair loss, weight reduction and any change on complete blood count. Lozano-Baena et al. [10] also reported cytotoxic effect of Borago officinalis with IC50 values of 0.28 mg/mL. Hamed and Wahid [11] also revealed that 150 mg/kg b.wt of Borago officinalis ethanolic extract is a safe dose.

Nepata cataria extract showed cell viability ranging from 34.36 to 83.67 per cent against the extract concentration of 5 to 100 μg/ml with IC50 (µg/ml) on HeLa cell line as-112.07. Per cent cell toxicity ranged from 16.33 to 65.64 against the extract concentration of 5 to100 μg/ml. Nepata cataria aqueous extract showed low cytotoxic effect and the study corroborates with Hussain et al. [12], who revealed that LD50 (µg/ml) of aqueous Napeta juncea equal to 88.1. Similarly aqueous fraction (AQF) of Nepeta praetervisa leaves extract has showed lowest anti-leishmanial activity with IC50 value <100 μg/ml [13].

Table 1: In vitro cytotoxicity study of herbal extracts on HeLa cell line

<table>
<thead>
<tr>
<th>Test extract</th>
<th>Parameters</th>
<th>Test concentration (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Fumaria indica</td>
<td>Per cent cell viability</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Per cent cell cytotoxicity</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IC50(µg/ml)</td>
<td></td>
</tr>
<tr>
<td>Adiantum capillus</td>
<td>Per cent cell viability</td>
<td>92.28</td>
</tr>
<tr>
<td></td>
<td>Per cent cell cytotoxicity</td>
<td>7.72</td>
</tr>
<tr>
<td></td>
<td>IC50(µg/ml)</td>
<td></td>
</tr>
<tr>
<td>Nepeta cataria</td>
<td>Per cent cell viability</td>
<td>84.36</td>
</tr>
<tr>
<td></td>
<td>Per cent cell cytotoxicity</td>
<td>15.64</td>
</tr>
<tr>
<td></td>
<td>IC50(µg/ml)</td>
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<tr>
<td>Levandula stoeches</td>
<td>Per cent cell viability</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Per cent cell cytotoxicity</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IC50(µg/ml)</td>
<td></td>
</tr>
<tr>
<td>Borago officinalis</td>
<td>Per cent cell viability</td>
<td>95.63</td>
</tr>
<tr>
<td></td>
<td>Per cent cell cytotoxicity</td>
<td>4.27</td>
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<tr>
<td></td>
<td>IC50(µg/ml)</td>
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</table>
In case of *Levandula stoeches* extract per cent cell toxicity ranged from 0 to 41.77 against the extract concentration of 5 to 100 μg/ml. *Borago officinalis* extract exhibited per cent cell toxicity ranging from 4.27 to 43.17 against the extract concentration of 5 to 100 μg/ml with IC50 (μg/ml) on HeLa cell line as 26.91. Similar results were recorded by Bonyadian et al. [14], who revealed that *Levandula essential* has inhibitory and lethal effect on the premastigotes form of *Leishmania major* in vitro.

**CONCLUSION**

From above study it can be concluded that aqueous extracts of *Levandula stoeches* and *Borago officinalis* exhibits highest cytotoxicity on HeLa cell line and their use for in vivo trails can be carried out only after conducting cytotoxicity trails on HeLa selective cell lines. Aqueous extracts of *Fumaria indica*, *Adiantum capillus* and *Nepata cataria* are safe after conducting cytotoxicity study on HeLa cell line and thus their use for in vivo trails can be carried out.

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**REFERENCES**


