Evaluation Of Antimitotic Activity Of Solanum Torvum Using Allium Cepa Root Meristamatic Cells And Anticancer Activity Using MCF-7-Human Mammary Gland Breast Adenocarcinoma Cell Lines

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Objective: An attempt has been made to evaluate the antimitotic activity of Solanum torvum. The studies were extended to human cells using MCF-7- Human mammary gland breast adenocarcinoma cell lines. Methods: Preliminary antimitotic screening was done using Allium cepa root tip assay. The herbal powder obtained from various plant parts-dry leaves, dry fruits and fresh fruits were extracted with various solvents. The antimitotic activity was analysed using Allium cepa root meristematic cells. Experiments were carried out with incorporation of folic acid in the extract. Folic acid inhibited the antimitotic activity of S.torvum extract. Findings: The results obtained were compared with methotrexate-a known anticancer drug. Extracts of S.torvum was found to be extremely effective in the prevention of cell proliferation of the mammary gland breast adenocarcinoma cell lines. Discussion: The pronounced antimitotic and anticancer activities of S.torvum was due to its potential antioxidant property especially by the key role of phytochemicals such as polyphenols, steroidal saponin glycosides, alkaloids and flavonoids. Active principle sterol has been separated by TLC. Conclusion: These findings suggest that the promising antioxidant properties of the plant could be exploited in herbal preparations against oxidative stress, ageing, Ischemic heart disease in dissolving thrombus, microbial infections, hormone replacement therapy (HRT) and cancer justifying their use in traditional medicine.

Keywords: Solanum torvum; Allium cepa; Anticancer; Mammary carcinoma fibroblast MCF-7 cell lines; Antimitotic.

INTRODUCTION

Cancer is essentially a problem of abnormal cell growth. Under the influence of chemicals, viruses and free radicals, normal cells are converted to masses that divide in an uncontrolled manner.[1] The cytotoxic effect of plant polyphenols is shown to be mediated through apoptosis. Considering the entry of these natural polyphenols to absorb proteins and metal ions, there is a probability that they can elicit apoptosis signals through various receptors or proteins. Apart from this, the antioxidants prevent the free radical attack on DNA by acting as scavengers of these free radicals. A number of polyphenols are topo-II positions, inhibiting topo I/II isomerases thus enhancing the DNA cleavage.[2]

Another possible mechanism of action reported for anticancer drugs is inhibition of DNA synthesis and thus prevention of cell division. Folic acid supplied from the diet is essential for the production of terahydrofolinic acid (THF). The conversion of folic acid to THF is carried out by an enzyme folate reductase. Anticancer drugs compete with folic acid for this enzyme thus restricting the production of THF required for synthesis of DNA and consequently for cell replication. Cells which do not have adequate production of THF eventually die.[3]

Plants have been used in traditional medicine for several thousand years.[4] The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine.[4] During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world.[5-12]

Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources.

Today according to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases.[13]

Solanum torvum belongs to Solanaceae (nightshade family) is a spiny herb or shrub 3-4 m tall found throughout the tropical parts of India and in Andaman. Leaves, Ovate, sinuate or bilobed, lobes shallow, rarely deep, flowers white, in dense lateral racemes, berries globose, smooth, yellow or orange-red, seeds smooth.[14] Whole plants, fruits, leaves and root of Solanum torvum are used as antispasmodic, hypotensive, antibacterial, antifungal, anticonvulsant, CNS depressant activity, antiviral, antiinflammatory, molluscicidal, elastogenic, mutagenic, insecticidal.[15-18] antimarial digestive, diuretic sedative, liver and spleen enlargement, haemostatic, antitussive and in rhagades.[19-26]

The antimitotic activity was screened using Allium cepa root meristematic cells which have been used extensively in screening of drugs with antimitotic activity.[21,22] The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cells of this region undergo repeated divisions. The fate of cell division is higher in this region compared to that of the other tissues. This region is called the meristamatic region (meristos: divided).[23]

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Received 19-09-2011; Accepted 28-11-2011
This division is similar to the above mentioned cancer division in humans. Hence, these meristamatic cells can be used for preliminary screening of drugs with anticancer activity. Even though doubts can be raised about extrapolation of results from plant tissue to animals and finally to humans, Khilman has noted that plant cells are 1000 times more resistant to colchicines which is a potent anti-carcinogen and which acts by inhibiting the microtubule formation. Thus, it is possible that chemicals that affect plant chromosomes will also affect animals.[24]

S. torvum extract is effective against A. cepa root cells; it will also have antimitotic effect against animal and human cells. To evaluate this hypothesis, it was thought worthwhile to evaluate the activity of the extract of S. torvum on MCF-7-Human mammary gland breast adenocarcinoma cell lines. Phytochemical evaluation plays an important role in the standardization of crude herbal drugs.[25,26]

**MATERIALS AND METHODS**

**Collection and processing of plant material:** The plant *Solanum torvum* was collected in Chennai, Tamil Nadu, India. The identification and nomenclature of the plant was based on The Flora of Presidency of Madras [27] and The Flora of Tamil Nadu Carnatic.[28] They were later verified at Botanical Survey of India, Southern Circle, Coimbatore, India. All the preserved specimens were deposited at the Herbarium of Entomology Research Institute, Loyola College, Chennai. The freshly collected leaves and fruits were washed and air dried in the shade at room temperature. Dried leaves and dried fruits were taken separately and powered for extraction. Fresh fruit was homogenized with solvent and then extracted. *Allium cepa* bulbs (red variety) were purchased from the local market and stored for the entire study.

**Chemicals:** Carmine stain and solvents were procured from Sigma Aldrich, New Delhi, India. Eagles minimal essential medium for cell proliferation, MTT, RPMI-1640 were purchased from LGC Promochem India Pvt. Ltd. Bangalore, India.

**Preparation of plant extracts:** The plant material (leaves and fruits) were air dried in the laboratory at room temperature. It was then powdered and extracted with hot water by boiling for 30 minutes to get the aqueous extract. The extract obtained was concentrated and dried under controlled temperature (60°C). The dried powder was successively extracted with other solvents. Aqueous and Organic extracts were prepared. The dried powder was successively extracted with 70% ethanol, then with chloroform. Finally it was concentrated and made up to particular volume. Extraction with each solvent was done in a water bath for 60min with a reflux condenser. Each time before extracting with the next solvent the marc was dried in an air oven below 50°C. Fresh fruit was homogenized with solvent and then extracted. Each extract was concentrated and evaporated to dry extract. Extracts of desired concentrations were prepared for further study using these dried extracts.

Antimitotic activity: This activity was evaluated using *A. cepa* root meristamatic cells. *A cepa* were sprouted in tap water for 48 hr at room temperature. The bulbs that developed uniform root were used for the experiment. These roots were treated with above prepared extracts of 10 concentrations. Water was used as medium/vehicle dilution. The different fractions used have been mentioned in Table 1. A blank with water was used as control. Methotrexate was used as a standard control. After 3hr of treatment, the root tips were fixed with fixing solution of acetic acid and alcohol. Squash preparations were made by staining the treated roots with acetocarmine stain. The mitotic index was calculated as

\[
\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100.
\]

The aqueous and organic extracts were also subjected to preliminary phytochemical characterization, which revealed the presence of the phytochemicals-alkaloids, phenols, flavonoids, sterol, saponin glycosides, reducing sugars, proteins, cardio active aglycones and cardinolides, saponin glycosides. Folic acid added to the solution of methotrexate, aqueous extract and organic extract of *S. torvum*. A similar
experiment was undertaken to find out the probable mechanism of action through which the extracts and methotrexate act. Squash preparations made as above from the treated roots were observed.

Cell proliferation assay: MCF-7- Human mammary gland breast adenocarcinoma cell lines were obtained from the American type culture collection and grown in the Minimal essential medium Eagles with L-glutamine and Earle’s Basal salt solution adjusted to contain 1.5g/liter sodium bicarbonate, 0.1mM non-essential amino acids and 1 mM sodium pyruvate and supplemented with 0.01 mg/ml calf insulin -90% and 10% fetal calf serum in a humidified atmosphere of 5% CO2 at 37°C.

The effect of S. torvum on cell viability and growth was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) colorimetric assay [using a commercially available kit]. The compounds were dissolved in water as stock solution (1000×) and then diluted with RPMI-1640 for cell culture experiments. All solutions were prepared fresh on the day of testing. Human mammary gland breast adenocarcinoma cells were seeded at a density of 1×10^4 per well in a 96 well plate.

After 24hr, fresh medium was added containing aqueous extract of S. torvum at concentration of 0.1×10^-4, 1×10^-3, 1×10^-2, 1×10^-1, and 10 mg/ml. After 4 days of incubation, in medium containing extract of S. torvum cells were further incubated for 4 hr with the metabolic substrate, tetrazolium to formazan salt solution adjusted to contain 1.5g/liter sodium bicarbonate, 0.1mM non-essential amino acids and 1 mM sodium pyruvate.

Statistical analysis: The data was subjected to statistical analysis using analysis of variance followed by appropriate post-hoc tests. P<0.05 was considered as significant.

RESULTS

Effect of aqueous extract of S. torvum on mitotic activity: Antimitotic activity of aqueous extract was comparable to the activity of methotrexate (Table 1). The activity of organic extract was less than that of the aqueous extract. A one way ANOVA showed that there was a significant effect of treatment on mitotic activity. Post-hoc analysis using the Newman-Keuls test showed that the activity of all the different extracts were significant when compared with water (control). The aqueous extract showed lowest mitotic index i.e. highest activity amongst all the different extracts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration mg/ml</th>
<th>% of Non-dividing cells</th>
<th>% Dividing cells</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Control)</td>
<td>---</td>
<td>74</td>
<td>26</td>
<td>1.00</td>
</tr>
<tr>
<td>Methotrexate (Standard)</td>
<td>0.1</td>
<td>57</td>
<td>38</td>
<td>0.58</td>
</tr>
<tr>
<td>Organic Extract of S. torvum</td>
<td>10</td>
<td>58</td>
<td>39</td>
<td>0.43</td>
</tr>
<tr>
<td>Aqueous Extract of S. torvum</td>
<td>100</td>
<td>53</td>
<td>42</td>
<td>1.00</td>
</tr>
<tr>
<td>Standard (Water)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Antimitotic activity after treatment of Allium cepa roots with aqueous, organic extracts of Solanum torvum and methotrexate

Effect of folic acid on antimitotic activity of S. torvum and methotrexate: Analysis of data using a 3-way ANOVA showed that there was a significant effect of the pretreatment with folic acid on the antimitotic activity of S. torvum and methotrexate. The mitotic index increased when folic acid was added to the aqueous, organic extracts of S. torvum and methotrexate solution which otherwise reduce the mitotic activity in the absence of folic acid. However, did not increase with increase in folic acid concentration suggesting that the effect was not dose-dependent. By comparing the mitotic index of methotrexate and S. torvum, it was observed that incorporation of folic acid increased the mitotic index significantly in case of methotrexate, but not so
in case of *S. torvum*. Post-hoc analysis of the data showed that folic acid inhibited the anti-mitotic activity of methotrexate to a greater extent as compared to *S. torvum*.

**Table 2:** Antimitotic activity after treatment of *A. cepa* roots with aqueous extract of *S. torvum* + folic acid, organic extract of *S. torvum* + folic acid and methotrexate + folic acid

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations (mg/ml)</th>
<th>% of non-dividing Cells</th>
<th>% of dividing cells</th>
<th>Mitotic Index</th>
<th>Mitotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid Methotrexate Aqueous extract Organic</td>
<td>0.1</td>
<td>0.1</td>
<td>----</td>
<td>----</td>
<td>23</td>
</tr>
<tr>
<td>extract of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. torvum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid Organic Extract of S. torvum</td>
<td>0.1</td>
<td>----</td>
<td>10</td>
<td>----</td>
<td>32</td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract of S. torvum</td>
<td></td>
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</tbody>
</table>
| P-Prophase, M-Metaphase, A-Anaphase, T-Telophase

**Plate 3:** Different stages of mitosis of *Allium cepa* roots after treatment with water

**Plate 5:** Antimitotic activity of organic extract of *S. torvum* and the stages of cell division

**Plate 4:** Antimitotic activity of aqueous extract of *S. torvum* and the stages of cell division

**Plate 6:** Normal MCF-7 Human mammary gland breast adenocarcinoma cell lines –showing confluent monolayer (20X magnification)
DISCUSSION

The result from the study showed that the aqueous extract of S. torvum had excellent anti-mitotic activity that was comparable to the activity of methotrexate. Maximum numbers of non-dividing cells were observed. Methotrexate-anticancer drug competitively inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis. Methotrexate acts specifically during DNA and RNA synthesis, and thus it is cytotoxic during the S-phase of the cell cycle. Logically, it therefore has a greater toxic effect on rapidly dividing cells such as malignant and myeloid cells.

The addition of folic acid inhibited the antimitotic activity of S. torvum significantly, but does not completely inhibit the activity of methotrexate.

Aqueous extract of S. torvum was also effective in reducing the cell viability of MCF-7. Human mammary gland breast adenocarcinoma cell lines that may be acting following the same mechanisms as those in the Allium cepa meristamatic cells. By virtue of this, if the extract is administered in humans it may prevent cell proliferation by directly combining with cell receptors/enzymes and eliciting signals or cell apoptosis. Phytochemical characterization of the different extracts revealed the presence of the phytochemicals: Indole alkaloids, polyphenols, flavonoids, sterol, saponin glycosides, reducing sugars, proteins, cardio active aglycones and cardinolides. Aqueous fraction contains steroids along with other polar constituents. Though, the probability of steroids extracted in a polar solvent was low, these steroids occurred as aglycones of the saponin glycosides after the glycosides hydrolyzed. There
Phytosterol has effect on apoptosis. The rate of tumor growth is dependent upon a balance between the rates of cell proliferation and apoptosis. Apoptosis is a programmed cell death, as influenced by phytosterol. Hence, the sterols from S. torvum must be contributing to the anticancer potential of the herb.

The aqueous extract of S. torvum seems to prevent prophase stage in cell division where DNA duplication occurs. Methotrexate is a known anticancer drug that inhibits DNA synthesis. When folic acid was supplemented to the methotrexate and the total aqueous extract, it was seen that the mitotic index increased. Thus, it may be suggested that the extract may be acting through the pathway inhibiting tetrahydrofolic acid and hence folic acid is required for DNA synthesis that arrest cell division. Methotrexate is known as anticancer drug which compete with folic acid for the enzyme reductase. The total aqueous extract of S. torvum may also be competing with folic acid thus inhibit the DNA synthesis. Hence, addition of folic acid increases the mitotic index due to the availability of folic acid. However, the mitotic index does not increase significantly in case of S. torvum as compared to that of methotrexate. This may be because the extract may be mediating its effects through other mechanisms also. The extract binding with different cell proteins which are responsible for cell division. This effect may be due to steroid glycosidic alkaloid or steroidal alcohol.

From this study, it could be suggested that S. torvum is a promising source of steroidal glycosidic alkaloid or steroidal alcohol, polyphenols, flavanoids, indole alkaloid and FRSA, which have the ability to modify the physiological function of cells and hence act as anti-cancer drugs to arrest the proliferation of cancer cells. The extract shows commendable antioxidant activity which also may be one of the contributing factors to its anticancer potential. The present need is to develop drugs that can potentially target cancer cells by means of their inherent difference to normal cells. The development of such drugs with differential action will be very valuable in cancer chemotherapy without the observed side effects. The methodology involves use of cancer cell lines to test the efficacy of the plant extracts in vitro. In the present study extracts of S. torvum was tested for the antitumor activity and it showed most effective inhibition of MCF-7. Human mammary gland breast adenocarcinoma cell proliferation. The study throws light on potential use of S. torvum in treatment of cancer.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared