Cytotoxicity study of Shalmali extract on human endometrial stromal cells

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ABSTRACT

Various herbs are extensively used by the customary medical practitioners for curing numerous diseases in their day-to-day practice. Shalmali (Bombax ceiba) is a recognized plant used in the customary system for the management of various diseases. It has an extensive range of pharmacological and medicinal applications. In the current study, efforts have been made to summarize diverse aspects of scientific studies on the role of Shalmali extract on uterine bleeding. Therefore the cytotoxicity study of Shalmali extract was performed on endometrial cell lines using MTT assay. The absorbance by human endometrial stromal cells (THESC cells) was noted and the percentage of cell viability of THESC with varying treatment concentration of Shalmali extract was calculated in contrast to the standard placebo. The Shalmali extract showed cytotoxicity effect on T HESCs cells and the IC50 of the extract is 110 μg/ml. To conclude that Shalmali extract showed a positive correlation was found to exist between the presence of antioxidant compounds and cytotoxicity against cells. Our results concluded that Shalmali extract could be used for the treatment of uterine bleeding.

INTRODUCTION

Uterine bleeding is one of the gynecological problems between females of the reproductive age group in quest of medical intervention (Albers et al., 2004; Whitaker and Critchley, 2016). Abnormal uterine bleeding (AUB) is a discrepancy in usual menstrual flow in terms of amount, duration, frequency, and interval in the menstrual cycle (Vollman, 1956; Bradley and Gueye, 2016). The signal and indication of abnormal excessive vaginal bleeding are given the term Asrigdara in Ayurveda treaties (Mehta et al., 2018). Extreme uncontrolled bleeding is one of the major gynecological complaints instigating admission in hospital and later surgical intervention due to emergency created due to prolonged continuous bleeding (Goldstein, 2019). There are numerous formulations stated in traditional practices to progress the quality of life and to decrease the percentage of surgical interventions in gynecological disorder (Balamurugan et al., 2018).

It has been demanded in Ayurveda that Bombax ceiba was commonly known as Shalmali, which possesses established medicinal properties and is an element of many formulations. Practically every part of this plant is used as a drug, and its flowers and roots are used for curing the many ailments (Chaudhary and Khadabadi, 2012). The root is sweet, stimulant, cooling, restorative, aphrodisiac, astringent, emetic, demulcent, and tonic. It is used in the management of diarrhea, menorrhagia, dysentery, and wounds. The gum is astringent, cool-
Table 1: This table depicts the MTT assay results of THESCs cells treated with Shalmali extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Conc. (µg/ml)</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THESCs untreated cells</td>
<td>-</td>
<td>0.487 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>THESCs + SE</td>
<td>10</td>
<td>0.412 ± 0.03*</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>25</td>
<td>0.385 ± 0.02*</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>50</td>
<td>0.312 ± 0.02*</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>100</td>
<td>0.281 ± 0.02*</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>200</td>
<td>0.175 ± 0.01*</td>
</tr>
<tr>
<td>7</td>
<td>Tranexamic acid (mg/ml)</td>
<td>10</td>
<td>0.104 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD (n=3); *P<0.001, as compared with THESCs untreated control. The IC50 of the Shalmali extract is 110.6 µg/ml.

Figure 1: Cytotoxicity results of THESCs cells upon Shalmali extract treatment.

Materials and Methods

Test Drug

The resin of Shalmali was obtained from the Indian Medical Practitioners’ Cooperative Pharmacy and Stores Ltd, Chennai, which is accessible with the brand name Shalmali Niryasa and used for the present study.

Preparation of sample

Dried powder Shalmali resin (15 grams) was weighed in a beaker for sample extraction with the help of ethanol and hydroethanolic solvents (20% ethanol: 80% distilled water) as solvents. The extraction was done with 100 ml of every solvent for a period of 48 hours. After extraction, the corresponding solutions were filtered and concentrated under abridged pressure and the extract was kept in refrigerator 4°C for further use.

Human endometrial Fibroblast cells

The THESCs immortalized cell lines procured from ATCC-CRL-4003. The cells cultured in the growth medium having equal proportion of Dulbecco’s modified Eagle’s medium and Ham’s F12 medium with 1mM sodium pyruvate, 3.1 g/L glucose and deprived of phenol red added with sodium bicarbonate (1.5 g/L), 90%; charcoal/dextran treated with 10% fetal bovine serum, 1% ITS and Premix 500ng/mL puromycin. The cells were kept in a 6% CO2, humidified incubator at 37°C. A thermometer was kept inside an incubator for providing a liberated readout of the culture temperature.

Cell viability by MTT assay

THESCs were cultured in 96-well tissue culture plates for the assay. The microplates filled with fibroblast cells (100 µl) with a density of 3×10^5 were taken as a negative control. The cells were allowed to observe for 24 hours, and the growth medium by means of micropipette and the monolayer of cells rinsed twice with MEM devoid of FBS for removing dead cells and extra FBS. 1ml of medium (without FBS) having various concentration of Shalmali extract (10-200 µg/ml) were added on to respective wells; 20 µl of MTT (5 mg/ml in PBS) was added on to every well, and the cells kept in 5% CO2 incubator for 6-7 hrs. After removing the medium, DMSO (1ml) was added on to standard well and Tranexamic acid (10mg/ml) was tested. Propanol (50 µl) was added to the wells and the plates were mixed gently to solubilize the molded formazan. The MTT arrives the cells and enters into the mitochondria where it is abridged to an
insoluble, colored (dark purple) formazan product. The plates were kept on a shaker for 15 min and the absorbance was measured on an enzyme-linked immunosorbent assay (ELISA) (MINDRAY90) reader at 570 nm. Each experiment was done in triplicate and the IC₅₀ of the test samples as the percentage survival of the cells was calculated.

Statistical analysis

Results were measured as mean ± S.E.M. Statistical significance was resolute by one-way analysis of variance (ANOVA) and post hoc least-significant difference test by SPSS software (version 22.0). P values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Cytotoxicity activity of Shalmali extracts was performed against T HESCs at various concentrations to find the IC₅₀ using MTT assay. Results of different concentrations of Shalmali extract, including 10, 25, 50, 100, 200 µg/ml, are tabulated in Table 1, and the cell viability percentage was graphically represented in Figure 1 and the values were *p<0.001 significantly different as compared with other T HESCs untreated cells. Ethanolic extract of Shalmali has significant cytotoxicity effect on T HESCs cells in a concentration range between the dose range 10 µg/ml to 200 µg/ml as compared with the negative control. Shalmali extract also exerts the higher cytotoxicity against THESCs cells in 200 µg/ml concentration. It was observed that the percentage of cell viability was decreased with increasing concentration of test compounds, the IC₅₀ value of Shalmali extract on T HESCs was 110 µg/ml by MTT assay (Table 1, Figure 1).

The current study was directed to examine the role of Shalmali extract on uterine bleeding. The intracellular decreasing power is mainly given by NAD(P)H that has been obtained from dehydrogenase activity in the endoplasmic reticulum, mitochondria and plasma membrane (Peng et al., 2005; Adan et al., 2016). The percentage of cell viability was observed to be decreasing with increasing concentration of Shalmali extract. So, the present study illustrates that the selected plant has significant cytotoxicity on T HESCs cells at 200µg concentration.

Tranexamic acid. Several literature studies showed that Shalimali retains astringent, stimulant, cooling, diuretic, demulcent, aphrodisiac, and tonic effects and also benefits in dysentery (Rameshwar et al., 2014). The therapeutic effect of Shalmali is partially due to the existence of flavonoids, sesquiterpenoids, bombamalosides, phenolics, shamimicin, bombamalones, bombasin 4-o-glucoside, bombesin, and bombalin (Jan et al., 2017). It is described to contain phytoconstituents like naphthal, polysaccharides, naphthoquinones, anthocyanins, lupeol, and shamimin, which plays a beneficial role in uterine bleeding problems.

CONCLUSIONS

New studies on pharmacological effects of Shalmali extract on uterine bleeding validate the effectiveness of the drug in ailments, which are used since prehistoric times and supports signs of beneficial use in Ayurveda. The existence of other interesting chemical compounds designates that Shalmali could serve as "lead" for the growth of new agents in disorders in the approaching years.

REFERENCES


