INTRODUCTION

An ulcer can be defined as a discontinuity in the skin, mucous membrane, or oral mucosa. Head-and-neck cancers are cancers that start in the tissues and organs of the head-and-neck region.[1] These include cancers of the throat, lips, mouth, salivary gland, and nose. Most types of head-and-neck cancer begin in squamous cells that line the moist surfaces inside the head and neck.[2] The study of traditional use of plants for medicinal use is known as ethnobotany.[3] There is a growing use of alternative and complementary anticancer medicines worldwide.[4] *Trigonella foenum-graecum* (fenugreek) is an annual plant and belongs to the family *Leguminosae*. It is the famous spices in human food. The seeds and green leaves of fenugreek are used in food as well as in medicinal application that is the old practice of human history. It has been used to increase the flavoring and color and also modifies the texture of food materials. It is known to have medicinal and therapeutic properties and is traditionally applied to treat disorders such as high cholesterol,[5] diabetes,[6,7] gastrointestinal ailments,[8] and wound inflammation.[9] Fenugreek seems to be a good source of bioactive compounds with not only anticancer but also several other pharmacological effects.[10] Scientists have reported several medicinal uses of fenugreek seeds and their protection against free radicals.[11] These protective roles are possible due to the non-nutritive secondary metabolites also known as phytochemicals. The major constituents that are present in fenugreek seeds are proteins, alkaloids, carbohydrates, lipids, fiber, flavonoids, vitamins, steroidal saponins, and nitrogen compounds which can be categorized under non-volatile and volatile constituents.[12] Fenugreek protective effect against breast cancer development was observed using the DMBA-induced mammary tumor model in rats.[13] It is of interest to explore the possibility of using phytochemicals as therapeutic agents.[14] Fenugreek has a mechanism of action which is similar to several anticancer drugs and is based on an ability to induce apoptosis.[15] The protein of fenugreek is found to be more soluble at alkaline pH.[16] The chemical constituents of fenugreek possessing anticancer activity are saponins and phytoestrogens.[17] Saponins can activate apoptotic programs which can lead to programmed cell death and selectively inhibit cell division in tumor cells.[18] A selective cytotoxic effect of fenugreek extract in vitro to a panel of cancer cell
lines has been observed, including T-cell lymphoma by Alsemarj et al.\textsuperscript{[19]} The seed powder in the diet decreased the activity of β-glucuronidase significantly and prevented the free carcinogens from acting on colonocytes. Mucinase helped in hydrolyzing the protective mucin. This was attributed to the presence of saponins, fiber, and flavonoids.\textsuperscript{[20]} In this study, we analyze the cytotoxic effect of fenugreek extract on the oral cancer cell lines (KB cell lines).

**Aim**

The present study is to analyze the cytotoxic potential of fenugreek extract on oral carcinoma KB cell lines.

**MATERIALS AND METHODS**

**Cell Culture**

KB cells were procured, placed in 25 cm\textsuperscript{2} culture flasks, and cultured in RPMI-1640 culture medium, with 10% fetal bovine serum, L-glutamine, 1% penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified CO\textsubscript{2} (5%) chamber and 95% air. The cells were detached using 0.25% EDTA trypsin. Neutralization of the trypsin was achieved using Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and PSGF, and cells were mechanically separated using a pipette. There were 96-well plastic culture plates filled with 200 μl of medium containing in each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO\textsubscript{2} and 95% air for 24 h to permit attachment of the cells to the plates.

**Cytotoxicity Assay**

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The KB cells were seeded at the density of 1 × 10\textsuperscript{3} cells/ml, were plated on into well plates, and treated with - Fenugreek extract (FGE) for 24 h. The cells were permitted to adhere for 24 h, and the growth medium (MEM) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS. Cell culture medium (DMEM) was used as negative control for assessment of cell viability. 1 ml of medium (without FBS) containing different concentration of drugs (100, 200, 400, 800, and 1000 μg/ml) were added in respective wells; 200 μl of MTT (5 mg/ml in phosphate-buffered saline) were added to each well, and the cells incubated for a further 6–7 h in 5% CO\textsubscript{2} incubator. After removal of the medium, 1 ml of DMSO was added to each well. The effect of the drug on cell growth inhibition was assessed as percentage cell viability, where vehicle-treated cells were taken as 100% viable. The supernatant was removed and 50 μl of propanol was added, and the plates were gently shaken to solubilize the formed formazan. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The plates were placed on a shaker for 15 min, and the absorbance was read on an enzyme-linked immunosorbent assay reader at 570 nm.

**RESULT AND DISCUSSION**

Cell viability changes by MTT was assessed in KB cell lines before and after incubating the cells with fenugreek extract. The standard drug cisplatin is taken as control. Cytotoxicity potential of fenugreek extract increases linearly with an increase in the concentration of the extract [Table 1, Graph 1]. Fenugreek is a common and less expensive substance. Used as an ayurvedic medicine in India. The anticancer potential of fenugreek should be extensively explored. The in vitro cytotoxic potential of fenugreek as a substance to degrade cancer cells points to the potential usefulness of fenugreek in the prevention and treatment of cancer.

![Graph 1: Cell viability changes](image-url)
Table 1: KB cells at different concentration along with control group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance 570 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB cells</td>
<td>–</td>
<td>0.489±0.25</td>
</tr>
<tr>
<td>FGE</td>
<td>100</td>
<td>0.406±0.03</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.315±0.12</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.287±0.06</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>0.198±0.05</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.104±0.16</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>10</td>
<td>0.096±0.11</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=3); *P<0.001, as compared with KB cells untreated. FGE: Fenugreek extract

REFERENCES