RESEARCH ARTICLE

In Vitro Cytotoxic Activity of Seed Oil of Fenugreek Against Various Cancer Cell Lines

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Abstract

In the present study, investigations were carried out to screen the anticancer activities of fenugreek seed oil against cancer cell lines (HEp-2, MCF-7, WISH cells), and a normal cell line (Vero cells). Cytotoxicity was assessed with MTT and NRU assays, and cellular morphological alterations were studied using phase contrast light microscopy. All cells were exposed toi 10-1000 µg/ml of fenugreek seed oil for 24 h. The results show that fenugreek seed oil significantly reduced the cell viability, and altered the cellular morphology in a dose dependent manner. Among the cell lines, HEp-2 cells showed the highest decrease in cell viability, followed by MCF-7, WISH, and Vero cells by MTT and NRU assays. Cell viability at 1000 µg/ml was recorded as 55% in HEp-2 cells, 67% in MCF-7 cells, 75% in WISH cells, and 86% in Vero cells. The present study provides preliminary screening data for fenugreek seed oil pointing to potent cytotoxicity against cancer cells.

Keywords: Cytotoxicity - cancer cells - fenugreek - cellular morphology

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Introduction

Cancer is one of the major causes of death in developed countries, together with cardiac and cerebrovascular diseases (WHO, 1998). Several methods exist for the treatment of cancer in modern medicine. These include chemotherapy, radiotherapy, and surgery. Chemotherapy is now considered as the most effective method of cancer treatment. Intervention with chemopreventive agents at the early stage in carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumors with chemotherapeutants severely affect the host normal cells. Hence the use of natural products now has been contemplated of exceptional value in the control of cancer.

Major resources scilicet natural products and synthetic compounds analog to known agents have been disclosed in possessing various bioactive compounds yearned by the drug development industries. The evaluation and the discovery of new anticancer agents is long-term process that encompasses many steps. The step broaches with the screening for anticancer properties, followed by the isolation and identification of bioactive compounds obliged to anti cancer properties, toxicity estimation of the isolated compounds and finally *in vivo* anticancer activity testing to verify the aptitude of the compounds (Vijayarathna and Sreenivasan, 2012). Further plant derived natural products have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer preventive effects. Natural products have been used as anticancer agents (Frei, 1982; Sasipawan et al., 2011), such as vincristine and vinblastine from Catharanthus roseus (Johnson et al., 1963), taxol and docetaxel from Taxus brevifolia (Wani et al., 1971) and camptothecins from Camptotheca acuminate (Wall et al., 1966). Even vegetables and fruits may help reduce the risk of cancer in humans (Chen et al., 2006; Moon et al., 2011).

Fenugreek (Trigonella foenum graecum) is an annual herb that belongs to the family Leguminosae. It has a long history as both a culinary and medicinal herb (Kaviarasan et al., 2006). The seeds of fenugreek are commonly used as a spice in food preparations due to the strong flavour and aroma. The seeds are reported to have restorative and nutritive properties (Khosla et al., 1995). Fenugreek seeds are used in remedies for diabetes and hypercholesterolaemia in Indian, Arabic and Chinese medicine. Its utility has also been proved experimentally in diabetic humans (Sharma et al., 1990). Hepatoprotective properties of fenugreek seeds in experimental models (Thirunavukkarasu et al., 2003; Kaviarasan and Anuradha, 2007) and antioxidant properties of fenugreek seeds against experimental cataract (Gupta et al., 2010) were also reported. In recent research, fenugreek seeds were experimentally shown to protect against breast (Amin et al., 2005) and colon cancers (Raju et al., 2006). Active components and their biological activities of isolates of the fenugreek seeds showing the antioxidant and anti-

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inflammatory activities have already been reported (Liu et al., 2012). Therefore, present study was designed to investigate the cytotoxicity of fenugreek seed oil against the four cell lines, i.e. Human epidermoid cancer cells (HEp2), human breast adenocarcinoma cells (MCF-7), human amniotic epithelial cells (WISH), and African green monkey kidney cells (Vero).

Materials and Methods

Chemicals and consumables

DMEM culture medium, antibiotics-atimycotic solution, fetal bovine serum (FBS) and trypsin were purchased from Invitogen, Life Sciences, USA. Consumables and culture wares used in the study were procured from Nunc, Denmark. Ethanol and all other specified reagents and solvents were purchased from Sigma Chemical Company Pvt. Ltd. St. Louis, MO, USA.

Cell Lines and cell cultures

Human epidermoid cancer cells (HEp2), human breast adenocarcinoma cells (MCF-7), human amniotic epithelial cells (WISH), and African green monkey kidney cells (Vero), were cultured in DMEM/MEM/RPMI-1640, supplemented with 10% FBS, 0.2% sodium bicarbonate and antibiotic/antimycotic solution (100x, 1ml/100 ml of medium). Cells were grown in 5% CO_2 at 37^{0}C in high humid atmosphere. Before the experiments, viability of cells was assessed following the protocol of (Siddiqui et al., 2008). Cells showing more than 95% cell viability and passage number between 12 and 18 were used in the present study.

Plant material and extractions

The fenugreek seeds used in this work were obtained from the local market of Riyadh, Saudi Arabia. The seeds were screened manually to remove bad ones. They were then dried to constant weight in an oven at 70^{0}C, ground using mechanical grinder, put in air-tight containers and stored in a desiccator. The oil from fenugreek seeds was extracted by continuous extraction in Soxhlet apparatus for 12 h using petroleum ether (60-80^{0}C boiling range) as a solvent according to the method described by AOCS (Horwitz, 1980). At the end of the extraction the solvent was evaporated. The oil thus obtained was dried over anhydrous sodium sulphate and stored -4^{o}C for further analysis.

Experimental design

Cells were exposed to various concentrations of fenugreek seed oil (10 µg/ml to 1000 µg/ml) for a period of 24 h. Following the exposures of fenugreek seed oil, cells were subjected to assess the cytotoxic responses using MTT assay, NRU assay, and cellular morphological alterations were studied.

Drug solutions

The extracts of fenugreek seed oil was not completely soluble in aqueous medium solution, therefore the stock solutions of all the extracts were prepared in Dimethylsulphoxide (DMSO) and diluted in culture medium to reach the desired concentrations. The concentration of DMSO in culture medium was not more that 0.1% and this medium was used as control.

Cytotoxicity Screening

MTT assay: Percent cell viability was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay as described (Siddiqui et al., 2008). Briefly, cells ($1x10^4$) were allowed to adhere for 24 h CO_2 incubator at 37°C in 96 well culture plates. After the respective exposure, MTT (5 mg/ml of stock in PBS) was added (10 µl/well in 100 µl of cell suspension), and plates were incubated for 4 h. Then, supernatants were discarded and 200 µl of DMSO were added to each well and mixed gently. The developed color was read at 550 nm using Multiwell Microplate Reader (Thermo Scientific, USA). Untreated sets were also run under identical conditions and served as control.

Neutral red uptake (NRU) assay: Neutral red uptake (NRU) assay was carried out following the protocol described by (Siddiqui et al., 2010). Briefly, after the respective exposure, the medium was aspirated and cells were washed twice with PBS, and incubated for 3 h in a medium supplemented with neutral red (50 µg/ml). Medium was washed off rapidly with a solution containing 0.5% formaldehyde and 1% calcium chloride. Cells were subjected to further incubation of 20 min at 37^{0}C in a mixture of acetic acid (1%) and ethanol (50%) to extract the dye. The plates were read at 540 nm using Multiwell Microplate Reader (Thermo Scientific, USA). The values were compared with the control sets run under identical conditions.

Morphological analysis: Morphological observation of cells treated with fenugreek seed oil were done to determine the changes induced by the fenugreek seed oil. All the cells were exposed to increasing concentrations (10-1000 µg/ml) of fenugreek seed oil for 24 h and cell images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 X magnification.

Statistical analysis

The results are expressed as mean and standard error of means (SEM). One way ANOVA was employed to detect differences between the groups of treated and control. The values showing $p<0.05$ were considered as statistically significant.

Results

Cytotoxicity assessment

The *in vitro* cytotoxic effect of fenugreek seed oil was evaluated by MTT and NRU assays. The percent cell viability in different cell lines observed by MTT and NRU assays are presented in Figures 1 and 2. The results showed that the seed oil of fenugreek significantly reduced the viability of all the cancerous cells in a concentration-dependent manner (Figure 1). Among the cell lines, HEp-2 cells showed the highest decrease in the cell viability, followed by MCF-7, WISH, and Vero cells by MTT assay. The cell viability at 1000 µg/ml were recorded 55% in HEp-2 cells, 67% in MCF-7 cells, 75%

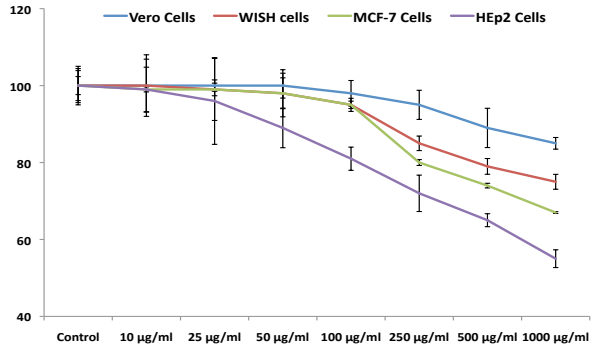

Figure 1. Effect of Seed Oil of Fenugreek on the Cell Viability of Different Cell Lines. Each cell line was treated with increasing concentrations (10-1000 µg/ml) of seed oil of fenugreek for 24 h, and the cell viability was determined by MTT assay

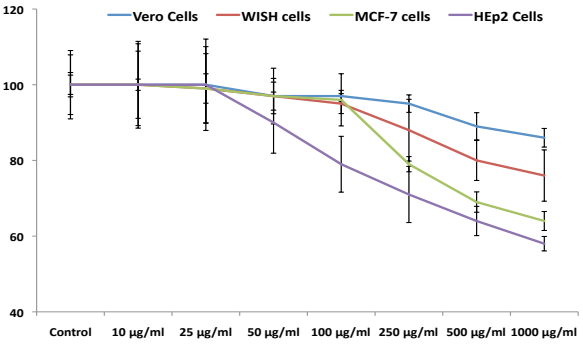

Figure 2. Effect of Seed Oil of Fenugreek on the Cell Viability of Different Cell Lines. Each cell line was treated with increasing concentrations (10–1000 µg/ml) of seed oil of fenugreek for 24 h, and the cell viability was determined by NRU assay

in WISH cells, and 86% in Vero cells (Figure 1). Similar kind of results was also found in all the cells exposed with various concentrations of seed oil of fenugreek by NRU assay (Figure 2). The results also showed that the seed oil of fenugreek significantly reduced the viability of all the cancerous cells in a concentration-dependent manner (Figure 2). The cell viability at 1000 µg/ml of seed oil of fenugreek were found 58% in HEp-2 cells, 64% in MCF-7 cells, 76% in WISH cells, and 85% in Vero cells (Figure 2). HEp-2 cells were found to be more sensitive and the cell viability was reduced maximum, followed by MCF-7, WISH, and Vero cells (Figure-2). Significant decrease of cell viability was only observed at 250 µg/ml and above concentrations of seed oil of fenugreek, and lower concentrations did not decrease the cell viabilities of cells (Figures 1 and 2).

Morphological changes

The morphological changes observed in various cell types are shown in Figures 3. Morphological alteration of HEp2, MCF-7, WISH, and Vero cells lines after the exposure of seed oil of fenugreek were observed under phase contrast microscope. The cells indicated the most prominent effects after exposure of the seed oil of fenugreek. Changes in morphology were found in concentration dependent manner. Cells exposed to 250

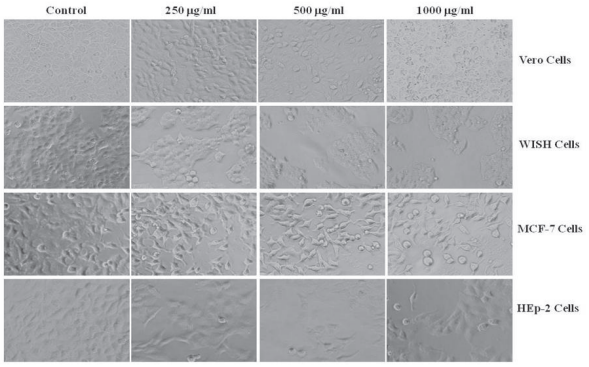

Figure 3. Morphological Changes after the Exposure of Various Concentrations (250-1000 µg/ml) of Seed Oil Of Fenugreek for 24 h. Images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20X magnification

µg/ml and above concentrations of seed oil of fenugreek for 24 h reduced the normal morphology of the cells and cell adhesion capacity in comparison to control (Figure 3). Most of the cells at 1000 µg/ml of seed oil of fenugreek lost their typical morphology and appeared smaller in size, shrunken and rounded (Figure 3).

Discussion

In this study seed oil of fenugreek was evaluated as new anticancer agent by using cytotoxicity end points i.e. MTT and NRU assays, and morphological alterations. Plants used in traditional medicines have been accepted as leads for therapeutic drug development in modern medicine. The seed oil of fenugreek was selected for this study, since fenugreek is commonly used as a spice in food preparations due to the strong flavour and aroma. The seeds are reported to have restorative and nutritive properties (Khosla et al., 1995), such as anti diabetic activity (Sharma et al., 1990), hepatoprotective activity (Thirunavukkarasu et al., 2003; Kaviarasan and Anuradha, 2007), and antioxidant properties against experimental cataract (Gupta et al., 2010). In recent research, fenugreek seeds were experimentally shown to protect against breast (Amin et al., 2005) and colon cancers (Raju et al., 2006). Thus, in order to understand the characteristic of the cytotoxicity effect of seed oil of fenugreek on cancer cells, four types of cell lines were chosen to study the cells i.e. HEp2, MCF-7, WISH, and Vero cells. The present study also demonstrated the cytotoxicity indices as a measure of percentage cell mortality calculated by MTT and NRU assays in the cells in a concentration dependent manner at the end of 24 h incubation with the seed oil of fenugreek. We found that seed oil of fenugreek significantly reduced the viability of all the cancerous cells in a concentration-dependent manner. Among the cell lines, HEp-2 cells showed the highest decrease in the cell viability, followed by MCF-7, WISH, and Vero cells. The data from the study agreed with the previous findings where they found difference in the sensitivity of the cell lines (Vijayan et al., 2004). They found that RD-228 cell line was more sensitive among HEp-2, RD-228 and Vero cells. The reduction in percent cell viability after 24 h of

treatment in the present study showed potent cytotoxic effects on various types of cancerous cells with seed oil of the fenugreek. This data is interesting as it suggests that the extract is more toxic for cancer cells than on normal cells (Das et al., 2010). Our results are also in well correlation with the previous findings in which the plant extract was found more cytotoxic to cancerous cells than the normal cells (Vijayarathna and Sreenivasan, 2010) due to the sensitivity of cancerous cells towards the death flavanoids (Das et al., 2010). Further the presence of alkaloids with flavonoids in Onobis hirta is also reported to show greater activity against cancer cells (Talib et al., 2011). Several alkaloids and these groups of compounds characterized by showing cancer inhibiting activity have been studied (Fawzy, 1994), and are effective in the treatment of some of the most common forms of brain and optic nerve tumors (Danna, 2006). The biologically active components present in fenugreek seeds having the anti-inflammatory and antioxidant activities have also been studied (Liu et al., 2012). The morphological changes in the cells were observed more prominent in treated cells showing extensive blebbing and vacuolation suggesting autophagic mechanism of cell death (Vijayarathna, 2012).

In conclusion, the findings from this study demonstrate the decrease in the cell viability of the cancerous cells exposed to seed oil of fenugreek. The present study also provides preliminary screening of the seed oil of fenugreek to have potent cytotoxicity against the cancerous cells.

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