Anti Leukemic Potential of Different Fenugreek Seed Germplasms

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Abstract
Drugs currently used in cancer treatment cause rigorous side effects, since the targeted pathways also occur in rapidly dividing normal cells. Newly emerging targeted drugs rely on inhibiting an aberrant tumor promoting pathway and the heterogeneity of cell types enable cancers to develop drug resistance. In addition, genetic instability enables malignant cells to evade inhibitor drugs by secondary mutations in target genes and other genetic changes. As a consequence, progress has been lacking in treating the majority of cancers. Plant constituents and derivatives hold great promise for cancer prevention and treatment. It is now believed that majority of cancers are attributed to environmental and dietary factors and incorporating fruits, vegetables, whole grains and spices in the diet seem to reduce the incidence of various cancers. The dietary agents are safe and readily available. However, application of plant components for cancer treatment/prevention requires better understanding of anticancer functions and elucidation of their mechanisms of action. The current study focuses on the anti-leukemic properties of fenugreek, a herb with proven anti-diabetic, antitumor and immune-stimulating functions. Here five fenugreek varieties of seed extracts were tested on B CLL (B Chronic Lymphocytic Leukemia) for its anti-leukemic potential. Results indicate that there is a good degree of potentiality of anti-leukemic activity of all these five germplasms though amber shoed the most potent activity as compared to other cultivars. This report showing fenugreek as an inducer apoptosis on B CLL cells hold a great promise in the future treatment of this form of leukemia.

Keywords: Fenugreek, Trigonella foenum graecum L., seed, germplasms, leukemia, anti leukemic

Abbreviations: ALL: Acute Lymphoblastic Leukemia; B CLL: Acute B Chronic Lymphocytic Leukemia; IC₅₀: half maximal inhibitory concentration; PI: propidium iodide

INTRODUCTION
Leukemias are a heterogenous group of neoplasms arising from malignant transformation of hematopoetic cells i.e. blood forming cells [4]. It is a disease of blood forming tissues in which the bone marrow is always involved and is characterized by overabundance of one cell type, usually an immature leukocyte. Leukemia is the most common childhood malignancy, representing 30 % of all childhood cancers in children under the age of 15 years [5]. In India and as well as in the rest of the world childhood Acute Lymphoblastic Leukemia (ALL) is the most prevalent form of leukemias [6]. Many anticancer therapies currently in use are inadequate not only in terms of their
therapeutic efficacy but also because they have undesirable side effects. On the other hand, certain dietary constituents known as phytochemicals have been shown to exhibit growth-suppressive activity and chemopreventive properties against various types of cancers [7] without the adverse side effects normally associated with current chemotherapies. Fenugreek (Trigonella foenum graecum L.) is commonly used as a traditional dietary ingredient in India and Egypt, among others. Fenugreek is believed to have originated in the North Africa area that is closest to the Mediterranean Sea. As a medicinal herb it has proven activity against hepatotoxicity [8], diabetes [9,10], hyperlipidemia [11] and cardiovascular diseases [9,12,13]. Fenugreek has anticancer properties [14] and has proven to be effective in preventing colon [15] and breast cancers [16]. Fenugreek extract also showed stimulatory effect on immune functions of mice [17]. More research on underlying molecular mechanism of the anticancer activity of fenugreek may lead to its development as an effective chemopreventive agent against various cancers. In this in vitro study we show, that fenugreek causes apoptosis on human B Chronic Lymphocytic Leukemia (B CLL) cells.

MATERIALS AND METHODS

Plant Material
Seeds of five different fenugreek lines (Fig. 1) with divergent origins grown in western Canada under dryland conditions were used for the study. Seeds were given by Dr. S N Acharya (Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada T1J 4B1)

Extraction
Crude extract was prepared from fenugreek seed’s of the above lines by separately soaking 1 g of seed in 10 ml distilled water overnight. The next day, the water, along with the extracted materials, was filtered, lyophilized (Lyolab BII LSL Seefroid Lyophilizer; Aclens-Lausanne, Switzerland) and the lyophilized material was stored at -20 °C for further use. The residue was re suspended in water or ethanol in concentrations as required.

Cell Culture
Freshly isolated CLL B-cells were re suspended at a density of 0.8-1.2 x 10^7 cells/ml in RPMI-1640 cell culture medium supplemented with 10 % heat inactivated foetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate (Invitrogen, Carlsbad, USA) and cultured at 37 °C in a humified atmosphere containing 5 % CO₂ [18].

Annexin V Staining
Freshly isolated CLL B cells were treated with different concentrations of fenugreek extracts. Cells were harvested and the percentage apoptosis was measured by flow cytometry (FACSVers, BD Biosciences, USA) after staining with fluorescein conjugated annexin-V and propidium iodide (PI) [2]. Five concentrations (5, 10, 25, 50 and 100 µg/mL) of crude seed extract were used on five different fenugreek germplasms. Chronic lymphocytic leukemia (n = 6) B cells were incubated with these crude extracts and then observed for the induction of apoptosis. Viable cells were scored as those that were negative for annexin V and PI. The percentage of apoptosis was calculated as the reduction in the number of viable cells between the treated and untreated samples. All reagents used in the experiment were of analytical grade and from BD Biosciences, USA.

RESULTS AND DISCUSSION
Fenugreek has primarily been introduced as a cholesterol-reducing plant, as well as an anti-hyperglycemic herb in humans and also
in laboratory animals. Fenugreek seeds have been found to contain protein, vitamin C, niacin, potassium, and diosgenin (a compound that has properties similar to estrogen). Other active constituents in fenugreek are alkaloids, lysine and L-tryptophan, as well as steroidal saponins (diosgenin, yamogenin, tigogenin, and neotigogenin). Some reports have described the overall stimulatory effect of Fenugreek on specific and non-specific immunefunctions of mice. Various herbal chemicals are extracted from Fenugreek. Fibers, flavonoids, polysaccharides, and saponins are the main chemical constituents of fenugreek. Apoptosis is a type of cell death, so called programmed cell death. In fact the agents capable of inducing apoptosis in tumors have the potential to be used for anti-tumor therapy. The apoptosis inducing activities of flavonoids have been identified in several studies. In this study we have tried to investigate the effect of Fenugreek extract on B CLL cells.

To explore the anti leukemic potential [apoptotic effect] of crude seed extracts of fenugreek on CLL B cells, we conducted flow analysis. We examined the cytotoxic effects of fenugreek seed extracts varying in concentrations from 0-100 mg/ml (48 h treatment) on the CLL B cells. A dose-dependent induction of apoptosis was observed in CLL B cells treated with fenugreeks seed extract (Fig. 1). Out of the five fenugreek germplasms, Amber and L3375 demonstrated robust anti-leukemic activity. Other lines, including Tristar, exhibited modest effect as far as apoptosis of CLL B cells is concerned [19]. The IC₅₀ (half maximal inhibitory concentration) values of Amber and L3375 were 25.2 and 29.5 mg/ml, respectively (Fig. 1). This preliminary study opens up the possibility of some compounds present in Western Canada grown Amber and L3375 seeds, which may be source of a potential drug for the treatment of CLL.

Figure 1. Induction of apoptosis on B CLL cells with five fenugreek germplasms (crude seed extracts) at 48 h of incubation as evidenced by Annexin-PI staining. Results are Mean ± Standard Deviation of three separate experiments.
CONCLUSION
In various areas of North Africa the fenugreek seed paste were traditionally eaten by women to gain weight in combination with sugar and olive oil. Fenugreek has a long history as a breast enlarger and contains diosgenin which is used to make synthetic estrogen [20]. It has been found to promote the growth of new breast cells and increase the size and fullness of the breasts. Of all the herbs used for breast enlargement fenugreek has the highest concentrations of the effective plant compounds [20]. It may reduce the amounts of calcium oxalate in the kidneys. Calcium oxalate often contributes to kidney stones. In animal studies, fenugreek also appeared to lessen the chance of developing colon cancer by blocking the action of certain enzymes. Our study conclude that fenugreek shows a great potential as a locally adapted cultivar suited to the dry land region of the prairies in western Canada and also in Africa for future nutraceutical use particularly in fighting against Chronic Lymphocytic Leukemia. It would have been of great use if fenugreek is cultivated in larger parts of Africa and its simultaneous inclusions in the dietary would have improved the heath condition of the people of Africa.

REFERENCES
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