

Ameliorative Effect of *Trigonella foenum graecum* (Fenugreek) Seeds Infusion on Mouse Lymphocytic Leukemia Cells Induced Ascities in Mice

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Abstract: Fenugreek (FG) or *Trigonella Foenumgraecum* was known to be rich in antioxidant compounds supporting its popular use in traditional medicine. Literature pointed that it can be used as anti -cancer . this work aimed to re-investigate the potential antineoplastic efficacy of water extract of fenugreek seed against a well- known animal model of Mouse Lymphocytic Leukemia (L1210) induced ascites in mice. Forty adult male mice divided into 4 equal groups. Group I: received 1 ml of normal saline and served as the control. Group II: maintained on the 20% (w / v) fenugreek seeds soaked in boiled water daily. Group III: received L1210 Leukemia cells and without treatment. Group IV: injected with L1210 tumor cells and received 20% (w / v) of boiling water infusion of the fenugreek seeds daily. Animal weight and rate of developing ascites were recorded. After 21 days all animals are euthanized, ascetic fluid was aspirated and measured. Abdomen was opened for exploration of visceral organ or tumor transformation to solid mass. Results revealed that nimals of GIII which were injected with L1210 cells developed ascites with rapid increase in weight. Abdominal exploration showed huge bloody ascites with transformation of leukemic cells into lobulated solid masses attached to visceral organs, muscles and overlying skin . There was damage of internal structure of organs invaded by tumor cells/. GIV: which received FG, showed less body weight, decreased amount of ascetic fluid and less tendency to solid tumor transformation. Examination of nearby organs revealed less tumor invasion with improved the morphological changes, reduced tissue damage and rebuilt of the surrounding visceral organs.In conclusions: Boiled water extract of FG exerted an anti -proliferative effect on L1210 cell line reducing ascites development and cancer transformation to solid mass.The mechanism by which it control cancer growth could be attributed to its high content of antioxidants, which to be confirmed needs further investigation. Fenugreek seeds extract could be promising s anticancer agent and should be tried in clinical field.

Key words: L1210 cells • Fenugreek • Seminiferous tubules • Kidneys • Testes • Mice

INTRODUCTION

Trigonella foenum-graecum (fenugreek) grows once a year, widely distributed throughout the world. Presence of phytochemicals renders fenugreek as one of the

important medicinal plants. The leaves and seeds been used extensively to prepare extracts and powders for medicinal uses [1, 2]. It demonstrates antioxidant impact in diabetes mellitus due to the existence of various ingredients [3, 4]. The protective roles of fenugreek are

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due to the non nutritive secondary metabolites phytochemicals [5]. The major constituents that are present in fenugreek seeds are carbohydrates, proteins, lipids, alkaloids, flavonoids, fibers, saponins, steroidal saponins, vitamins and minerals, nitrogen compounds which can be categorized under nonvolatile and volatile constituents [6].

L1210 are mouse lymphocytic leukemia cells which are derived from the ascitic fluid of 8-month-old female mice. While they are lymphocytic B-cells they are more like lymphoblast in morphology. Cells of ascitic transplantable tumors, such as Ehrlich ascites tumor or leukaemia L1210, can be propagated in vivo by growing them as cell suspensions in the intraperitoneal cavity of mice where the individual cells are suspended in an ascitic fluid and do not adhere to the peritoneal membrane [7-9].

The chemical constituents of fenugreek possessing anticancer activity are phytoestrogens and saponins [10, 11]. Saponins selectively inhibit cell division in tumor cells and also can activate apoptotic programs which can lead to programmed cell death [12], inhibited the proliferation of cells along with the induction of apoptosis. The effect on apoptosis can be validated by observing the effect on apoptotic proteins. Shishodia and Aggarwal reported that through inhibition of tumor necrosis factor [13]. Protodioscin, a furostanol saponin isolated from fenugreek, also induces apoptotic changes leading to death in a leukemic cell line [14].

Supporting the popular heritage medically, the current study aimed to elucidate the protective role of water extract fenugreek seed against the growth of Leukemia L1210 cells induced ascities in experimental mice.

MATERIALS AND METHODS

Animals: Experiments were performed on forty, 10-weeks old male mice weighted 30-40 grams, bred in the animal facilities of King Fahd Medical Research Center (KFMRC), King Abdulaziz University (KAU), Jeddah, Saudi Arabia, under a 12h light/dark cycle at a temperature of 25°C and relative humidity ranging from 60 to 70% throughout the experiment. They were given standard pellet diet and water ad libitum and kept for two weeks to acclimatize to the environmental conditions. The protocol met the approval of the Institutional Animal Care and Use Committee at King Abdulaziz University. The use of experimental animals was conducted in strict compliance with the rules and regulations established by the Research Ethics Committee at KAU.

Preparation of Seed Extracts: The fenugreek seeds used were purchased from the local market. Dried seeds (200gm) were mixed with 1000 mL of boiled distilled water (100C) and the mixture was left for 5 minutes, cooled and filtrated and used in replacement of normal water.

Experimental Design: Animals were randomly divided into 4 groups (10 mice in each).

GI: Normal control mice fed normal mice pellets and water ad libitum and received intraperitoneal injection of normal saline, 0.9% NaCl

GII: Mice were maintained on the 20% (w / v) of the previously prepared FG drink . daily and was served as a control positive for experimental treatment groups.

GIII: Included mice injected with L1210 tumor cells and without treatment and serves as negative for experimental treatment groups.

GIV: Includes injected mice with L1210 tumor cells and received 20% (w / v) of water soaked fenugreek seeds daily as replacement to normal water.

Induction of Cancer and Model: L1210 cells (0.2 ml) suspended in serum-free growth media (MSF / mouse or 200 microliter / mouse (1 × 510 cells / ml) was intraperitoneally inoculated by injecting a single dose 0.2ml of a 5% dilution of ascites tumor in 0.9% NaCl to the mice of GIII. The health and behavioral status of the mice in all groups was followed up as well as the average weights of mice per week have been recorded in the four groups.

Preparation of Tissues for Histopathology: Tissue samples were taken from the solid cancerous mass and organs that the tumor creeps to it as testis, kidneys, surrounding skeletal muscles and overlying skin of GIII and GIV at three weeks after treatment. The samples were fixed in formol saline, processed through graded alcohols and xylene and embedded in paraffin blocks in automatic processor of the pathology lab of King Abdulaziz University Hospital. Serial sections of 4-6 μ were made on longitudinally and transversely oriented specimens. Sections were routinely stained with Hematoxylin and Eosin and examined microscopically according to Bancroft and Gamble [15].

Statistical Analysis: The results are expressed as Mean ± Standard Deviation (SD). Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS)

software package for Windows. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD).

RESULTS

General Observation of Animals: Animals of GII: received fenugreek seed water extract looked healthy, active and energetic. Animals injected with L1210 (G111) showed signs of massive ascites, with increased body weight. The animals looked weak, anorexic and died after 3 weeks of L1210 injection. Leukemic animals in GIV which received water extract of fenugreek seeds, appeared energetic and of good general health with less accumulated ascitic fluid. Deaths in the latter group occurred late after 10 weeks of L1210 injection and administration of water extract of fenugreek seeds.

Body Weight: GIII mice received L1210 showed significant increase in body weights due to accumulated ascitic fluid in comparison the other groups. During dissection it was noticed that the injected L1210 was transformed into hardwood cancerous masses, which appeared as large coherent blocks with irregular white grainy surface, friable; and stuck to most visceral organs, as well as the skin and muscles, causing severe damage. In GIV, administration of FG water extract limited the ability of cancer cells L1210 to form solid masses or invasion into nearby organs.

Histopathological Examination of the Solid Cancerous Masses: Injection of L1210 leukemic cells by intrperitoneal route into male mice result in formation of

solid cancerous masses. They looked highly cellular and surrounded by congested blood capillaries. The nearby peritoneum showed infiltration by tumor cells. Tumor cells were seen invading nearby vessels (Fig. 1). Magnified power showed that tumor masses showed narrow central necrosis. Cells showed malignant criteria including hyper chromatic nuclei and numerous mitotic figures (Figs. 2A, Band C). Administration of FG water extract limited the ability of cancer cells L1210 to form solid masses or invasion into nearby organs. Cancerous cells looked shrunken leaving clear halo around them. The nuclei are small, irregular or deformed, dark and degenerated (Fig. 2 D).

In the untreated L1210 group the solid tumor attached to underlying muscle showed massive cellular proliferation, muscle fibers looked swollen with decreased staining beside presence of malignant cells within muscular capillaries. While in treated group, tumor masses showed marked decrease in cell proliferation. Tumor cells appeared shrunken, small-sized dark color nuclei. There was less tendency to invade muscular capillaries (Fig. 3). An extension to overlying skin with ulceration were observed (Fig. 4).

In untreated group cancerous cells were seen invading the testis resulting in marked toxic damage to seminiferous tubules germ cells and disappearance of sperms in addition to the spread of the tumor to the epididymis which showed disorganization and loss of the typical cyto-architecture of the tubules (oval or circular) (Figs. 5A, B&C). FG treated group showed decrease in cancer mass, intact seminiferous tubules and marked decrease in cancer cell invasion to epididymal components.

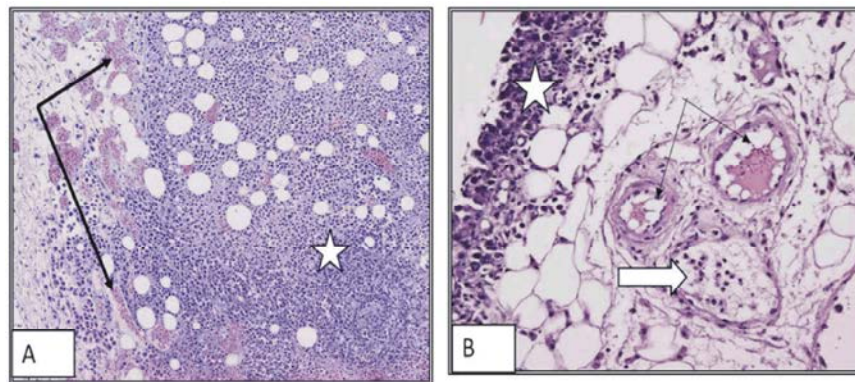


Fig. 1: Solid cancerous masses developed after injection of L1210 showing :A: highly cellular mass (white star) with peripheral congested blood capillaries (black arrows) B. Nearby peritoneum rich in fat cells showed infiltration by tumor cells (white star). Blood vessels showed slight perivascular fibrosis. Tumor cells were seen within lymphatic vessel (white arrow x 40)

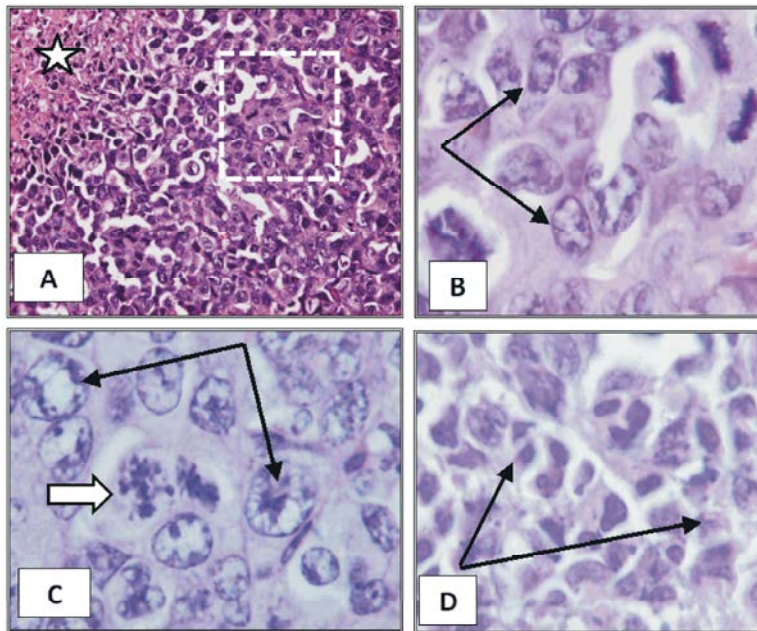


Fig. 2: Sections in untreated L1210 solid mass showing A: part of the mass with central necrotic area (star) and highly cellular periphery (dotted square), B. Pleomorphic highly euchromatic nuclei(black arrows) and numerous mitosis (white arrow). C: another field showing highly active cells with pleomorphic euchromatic nuclei and large nuclear/ cytoplasmic ratio (black arrows) and abnormal mitotic figures(white arrow). D.FG treated tumor showing atrophy of L1210 tumor cells. They looked shrunken leaving clear halloo around them. The nuclei(black arrows) are small dark and degenerated (H&E stain x 20)

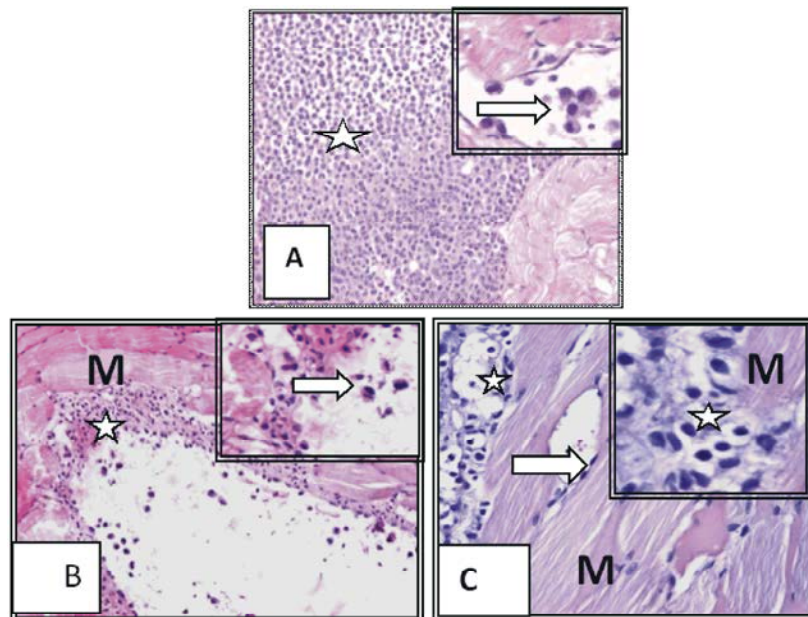


Fig. 3: Sections in untreated solid L1210 solid tumor with massive cellular proliferation(star) attached to muscle fibers which looked swollen with decreased staining(M). Insert showed malignant cells within muscular capillaries (white arrow)x20). B: section from Fenugreek water extract treated animals showing degenerated cancer cells with decreased tumor mass. Cells within capillaries looked degenerated (white arrow) (x20). C: Magnified power to show deformed degenerated cancer cells (stars) compared to non treated group(A). Muscle fibers looked also more preserved (× 40.)

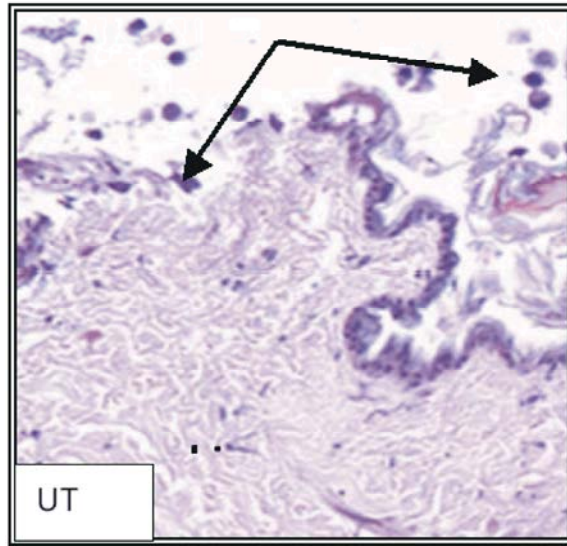


Fig. 4: Spread of cancerous cells (arrows) to ulcerating abdominal skin layers overlying tumor mass (H&E stain x20)

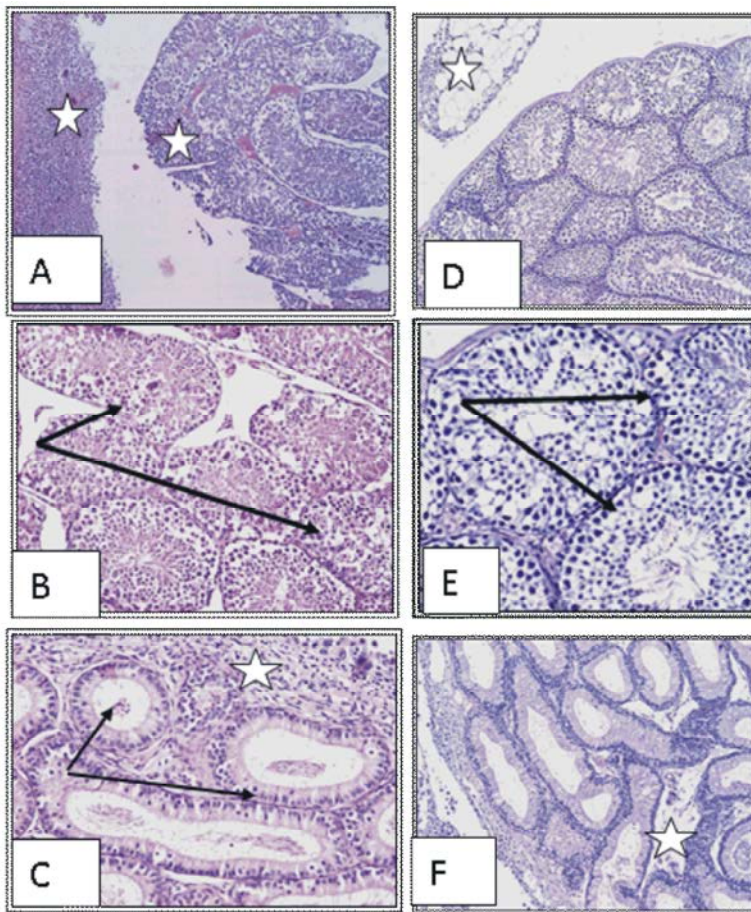


Fig. 5: Histological changes of the testis of L1210 injected mice of untreated group showing: A. Spread of cancerous mass (star) into testicular tissue B. marked destruction of seminiferous tubule germ cells (arrows) C. invasion (star) between epididymal tubules (arrow) D-F. FG treated group showing decrease in cancer mass (star) intact seminiferous (B) and epididymal tubules (arrows) with marked decrease in cancer cell invasion (H&E stain x20)

DISCUSSION

This study was performed to evaluate the beneficial and protective role of water extract fenugreek seed against the growth of Leukemia L1210 cells induced ascities in experimental mice. Results clearly indicate improved the morphological changes, reduced tissue damage and rebuilt of the surrounding visceral organs and this coincides with the findings of a prior research [16]. Several studies on anticancer properties of chemical constituents of fenugreek have been done and have shown positive results. Some constituent of alkaloids, called "trigonelline," has revealed potential for use in cancer therapy [11].

The reported phytochemistry of fenugreek and its pharmacological uses. Because of its medicinal effects, many scientists consider fenugreek as a potential nutraceutical. The clinical uses of fenugreek can be attributed to the rich chemical constituents it possesses. These chemicals make it a strong candidate in every domain as they help in alleviating dependence on synthetic drugs as well as other expensive treatments to cure diseases [16].

Administration of L1012 in the present study produced atrophy, vacuolations, cellular debris and the seminiferous epithelium was sloughed off at many points. a wide interstitium and congested capillaries and homogeneous oedematous material in the interstitium were noticed. These changes are consistent with the findings of Heba and Abd-Elghany [18, 19]. Degenerative alterations in the testicular tissues with damage of the supporting cells of Sertoli that ultimately causes irreversible loss and detachments of spermatogonia were also noticed and these agreed with a previous research [16, 20].

Solid tumors develop when a mouse is injected with a suspension of these cells intraperitoneally. Mice bearing ascitic in the intraperitoneal cavity survive 22 (± 2) days, whereas animals with fenugreek seed survive over 40 days, with a great dispersion of the survival time of individual specimens. This clearly indicated that the water solution of fenugreek seed extract showed the antineoplastic effect against ascites carcinoma cells in mice. Oral administration of the extract resulted in change in number and growth pattern of ascites cells and tumor growth was also seemed to be significantly inhibited improved the morphological changes, reduced tissue damage and rebuilt of the surrounding visceral organs [21].

Devasena & Venugopal [22]. observed that fenugreek seeds in the diet inhibited colon carcinogenesis. This was attributed to the presence of fiber, flavonoids and saponins. Inhibition of the mammary hyperplasia and decrease in its incidence were seen in rat after daily aqueous seed extract of fenugreek was given and could be attributed to cytostatic and cytotoxic effect of fenugreek seed extract in breast cancer in the mammalian model [23]. Ethyl acetate extract of the fenugreek seeds had a significant hypocholesterolemic and demonstrated a preventive effect on fat accumulation and dyslipidemia effect and antioxidant activity in cholesterol-fed rats [24, 25].

The ameliorating effects of fenugreek in the current work were clear and these results are consistent with the less or no alterations in the mice testes and other visceral organs as a result of L1012 injection. Similar findings were reported by Sen Gupta *et al.* [26]. The present study illustrated that the water extract of fenugreek seeds has been an inhibitory effect on growth cancer cells of L1210 type, which notes the apparent atrophy of the cancerous lumps in the viscera, as well as less or no ascities. In spite of the extension of the cancer cells from the peritoneal cavity to the surrounding organ but it appeared scattered and did not turn into a coherent tumor.

The presence of a large number of macrophages and giant cells in the tumor area which indicates the importance of fenugreek seeds extract as a catalyst of the immune system in an attempt to eradicate the tumor. Devasena & Venugopal. [22] attributed the anti-cancer activity of extract of fenugreek seeds to the antioxidant flavonoids in them such and saponins. In GIV, in spite of the extension of the cancer cells of the peritoneal cavity membranes of surrounding organ but it appeared scattered and did not turn into a adherent tumor, histological examination did not show any evidence of spread within the blood vessels or organs as the testes showed normal architecture of the seminiferous tubules. The masses apparent atrophy of cancer cells and the absence of the congestion of blood vessels of the tumor area compared to the GIII [21].

CONCLUSIONS

It is clear that from the obtained results the effectiveness of the water soaked seeds delay the onset of cancer, able to reduce the amount of ascetic fluid and prolongation of life in mice injected with leukemia L1210 cells.

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