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Effect of germination on anticancer activity of *Trigonella foenum* seeds extract

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ABSTRACT:

Trigonella foenum has a role in cancer treatment. This study tries to evaluate the effect of germination on the anticancer activity of fenugreek seeds. We prepared a dry and germinated aqueous extract from fenugreek seeds. We investigated the growth inhibitory effect of both extracts on MCF7 human breast and Pancreatic (AsPC-1) cells. Fenugreek extracts induced significant effect on cell viability, increased caspase-3 & 6 concentrations and LDH activity and caused nucleosomal DNA fragments. In summary, our results indicated that both dry and germinated extracts induced apoptosis in both cell lines. The effect of germinated extract was higher than that of the dry extract. This can be explained as germination increased the antioxidant characters of the extract. Germination also increased the phytochemical components (flavonoids, tannin, steroids, alkaloids, phytate, phenolics and trigonellin) of the extract which are believed to have anti-tumor activity.

1. Introduction

Trigonella foenum plant is named as fenugreek in English and "helba" in Arabic (Hammiche and Maiza, 2006). This plant possesses several pharmacological activities (Yadav and Baquer, 2014). The antitumor activity of *Trigonella foenum* dry seeds extracts and compounds isolated from it is well documented. The aqueous extract obtained from the dry seeds was cytotoxic toward several cancer cell lines T-cell lymphoma, B-cell lymphoma, thyroid papillary carcinoma, and breast cancer (Alsemari et al., 2014; El Bairi et al., 2017). In recent years, increasing attention focused on agents present in dietary and herbal plants as their bioactive substances to be used as anticancer agents (Hammiche and Maiza, 2006).

Germination induces the activation many proteolytic and lipid degrading enzymes that help improve protein digestibility as well as fat absorption capacity and increase the antioxidant capacity and the phytochemical components content of the seeds (Naguib, 2019).

Most researches on the anticancer activity of the fenugreek were on the dry seeds extract (Raju et al., 2004; Arlt et al., 2013; Alsemari et al., 2014; Alshatwi et al., 2017; El Bairi et al., 2017). From this point of view we aimed in this study to study the effect of germination on the anticancer activity of fenugreek seeds extract on human pancreatic cancer cell line (AsPC-1) & breast cancer cell line (MCF-7 cell line). We aimed to increase the anticancer activity of the fenugreek seeds with germination and study the mechanism of the fenugreek extracts as anticancer agent.

2. Materials and methods

2.1. Extract preparation

The fenugreek seeds were obtained from Egypt local market. The seeds were botanically authenticated with voucher specimens that were deposited in the Cairo University Herbarium, Egypt. The seeds were divided into two groups for extract preparation. The first remained dry (as it is obtained from the market) and the second was germinated on wet cotton piece for 48 h at 28 °C. The germinated and the dry seeds were dried in oven at 60 °C till constant weight to get rid of excess water. For the aqueous extract 10 g fenugreek seeds dry and germinated were grinded in 100 ml water. The homogenates remained for 30 min at room temperature; the extracts were centrifuged (10 min, 4000 rpm) and then the supernatant was filtered through a Whatman filter paper No. 1. After that the extracts were dried with rotary evaporator at 60 °C. The residue were dissolved in 10 ml distilled water and stored in refrigerator until administration. The obtained dry and germinated extracts were stored at 4 °C.

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2.2. Extract analysis

2.2.1. Antioxidant characters

The total antioxidant capacity of the extracts was evaluated by the phospho molybdenum method according to the procedure described by Prieto et al. (1999). Fenugreek extract with a volume of 0.3 ml was mixed with 3 mL of reagent solution (28 mM sodium phosphate, 0.6 M H_2SO_4 and 4 mM molybdate ammonium). Tubes containing the reaction solution were incubated at 95 °C for 90 min. Then, the absorbance was measured at 695 against blank (Dist. water (0.3 mL)). The total antioxidant activity is calculated according to the following equation:

$$\text{Total antioxidant capacity} = [(\text{Sample absorbance} - \text{Blank absorbance}) / \text{Blank absorbance}]*100$$

Reducing power assay was performed by the ferrocyanide method of Oyaizu (1986) as following One ml of test sample solution was mixed with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide. The mixture was incubated at 500 °C for 20 min. 2.5 ml trichloroacetic acid was added to the mixture. It was centrifuged at 3000 rpm for 10 min. 2.5 ml. The upper layer of solution was mixed with the same of distilled water and 0.5 ml of a freshly prepared ferric chloride solution. The absorbance was measured at 700 nm. The reducing power is calculated according to the following equation:

$$\text{Reducing Power} = [(\text{Sample absorbance} - \text{Blank absorbance}) / \text{Blank absorbance}]*100$$

Super oxide Dismutase (SOD) activity was measured by the nitro blue tetrazolium (NBT) reduction method (Beyer and Fridovich, 1987). The assay mixture comprising 3 mL of assay buffer, 60 µL of crude enzyme and 30 µL of riboflavin were incubated in illuminated an aluminum foil lined box containing two Fluorescent lamps at 25 °C for 7 min. After reaction, the absorbance of the blank solution and reaction solution was measured at 560 nm. SOD activities were calculated according to the following equation:

$$\text{SOD activity}(\%) = (1-A/B)\times 100 (\text{A: absorbance of sample; B: absorbance of blank})$$

.Catalase (CAT) activity was measured according to Kar and Mishra (1976). Polyphenol oxidase (PPO) activity was determined according to Beyer and Fridovich (1987). Peroxidase (POX) was measured according to Upadhyaya et al. (1985).

2.2.2. Phytochemical components

The tannin, glycosidic cyanide, steroid, saponin and alkaloid contents of the extract determination was performed according to Harbourne (1973) and modified by Trease and Evans (1996). Phytate content was estimated according to Vaintraub and Lapteva (1998). Total flavonoid content was measured with the aluminium chloride colorimetric assay (Pallab et al., 2013). Phenolic content of the extract was determined by the method of Julkunen-Tiitto (1985). Trigonelline content was determined according to Jyothi et al. (2017).

The increase ratio (%) was calculated according to the following equation:

$$\text{Increase ratio}(\%) = \frac{\text{Content in the germinated seeds extract} - \text{Content in the dry seeds extract}}{\text{Content in the dry seeds extract}} * 100$$

2.3. Cell line

Pancreatic (AsPC-1), Breast (MCF-7) human cancer cell lines were obtained from the tissue culture unit of the Holding Company for Biological Products and Vaccines (VACSERA),Giza, Egypt and supplied through the American Type Culture Collection (ATCC), cultured in a 37 °C incubator with 5% CO2 according to ATCC protocols.

Table 1
Antioxidant characters Total antioxidant (%), Total reducing power (%), SOD (%), CAT (µM H_2O_2 destroyed/min/g dry wt), POX and PPO Change in OD/min/g dry weight in Dry or geminated Fenugreek seeds extract.

Parameter	Dry	Germinated	Increase ratio (%)
Total antioxidant capacity	25.3 ± 0.9	69.0 ± 1.3*	172.9
Total reducing power	38.7 ± 0.7	70.4 ± 1.389*	82
SOD	12.3 ± 0.9	29.3 ± 0.289*	137.8
POX	0.0013 ± 0.0002	0.0029 ± 0.0009*	123.1
CAT	14.9 ± 1.03	34.9 ± 0.982*	133.8
PPO	0.0011 ± 0.0002	0.0024 ± 0.0003*	118.2

Values are given as means of 3 replicates ± standard error. Within a column, Means followed by asterisks are significantly different according to paired-samples *t*-test.

Table 2
Phytochemical components Total alkaloid, total tannin, total saponin, total steroid, total glycosidic cyanide, total phytate, trigonelline content (µg/g dry wt), total phenolic (mg pyrogallol/g dry wt) and total flavonoid (mg quercetin/g dry wt) in dry or geminated Fenugreek seeds extract.

Parameter	Dry	Germinated	Increase ratio (%)
Total alkaloid	45.9 ± 1.1028	77.5 ± 0.992*	68.8
Total flavonoid	12.9 ± 0.952	21.9 ± 0.983*	70.1
Total phenolic	29.4 ± 0.992	53.1 ± 0.283*	80.7
Total steroid	5.7 ± 0.7211	8.2 ± 0.152*	45.2
Total saponin	7.5 ± 0.3482	16.6 ± 0.672*	120
Trigonelline content	9.4 ± 0.6478	18 ± 0.093*	90.6
Total tannin	20.5 ± 0.937	55.9 ± 0.937*	173.2
Glycosidic cyanide	53.5 ± 0.882	95.5 ± 0.278*	78.5
Phytate	3 ± 0.280	5.4 ± 0.998*	84.1

Values are given as means of 3 replicates ± standard error. Within a column, Means followed by asterisks are significantly different according to paired-samples *t*-test.

2.4. Growth inhibition

The cell viability test was carried out using MTT assay as described by Mosmann (1983).

2.5. Determination of Caspase-3 and caspase-6 concentration

Caspase-3 level was determined using ELISA kit [Cat No. MBS261814], Abnova company Taiwan. Also, Caspase-6 level was demonstrated using ELISA Kit [Cat No. KA2638], Abnova company, Taiwan. According to manufacturer's instruction provided with the kits (Harrington et al., 1985).

2.6. Determination of LDH activity

The LDH activity was measured by kit (Takara Biochemicals, Otsu, Japan).

2.7. DNA fragmentation in MCF-7 and AsPC-1 cell lines

DNA fragmentation is measured according to Matassov et al. (2004) as following: cells were cultured and then treated for 24 h with different concentration of both dry and germinated fenugreek extracts. Adherent cells were separated by adding 1 ml Trypsin/EDTA. Cells were collected and centrifuged at 15,000 rpm for 5 min. Supernatant was removed and the cells were re-suspended in 10 ml ice cold phosphate buffered saline. Cells were centrifuged at 15,000 rpm for 5 min, and then supernatant was discarded. Cells were re-suspended in 1 vol digestion buffer (10 mM EDTA, 50 mM Tris-HCl, pH 8.0, 0.5% SDS, 0.5 mg/mL proteinase K). For 3×10^7 cells (Counting cells using a hemocytometer),

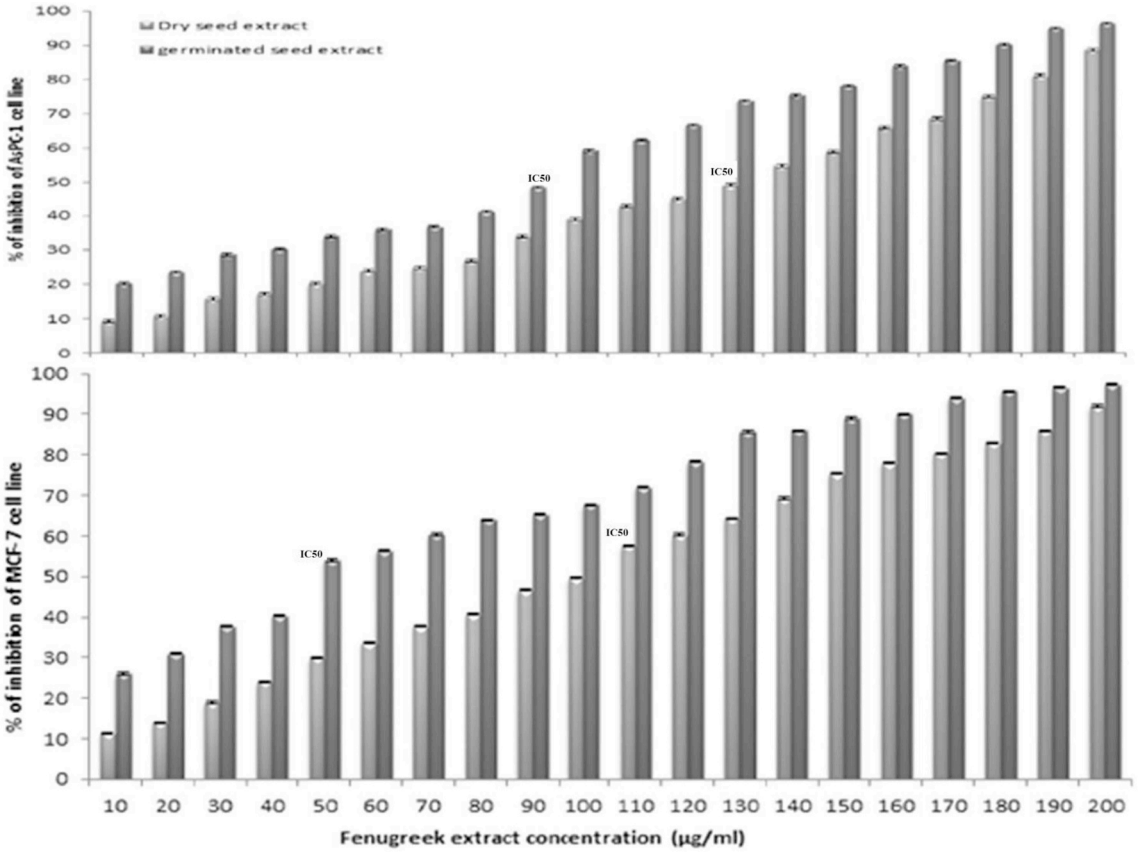

Fig. 1. Inhibitory effect of dry and germinated seed fenugreek extract on MCF-7 and AsPC-1 cell line.

Table 3
Mean level of caspase-3 concentration (ng/ml) in both MCF-7 and AsPC-1 cells in response to treatment with dry and germinated fenugreek extracts.

Conc (µg/ml)	Dry seed extract		Germinated seed extract	
	MCF-7	AsPC-1	MCF-7	AsPC-1
control	1.239 ± 0.001	1.143 ± 0.001	1.239 ± 0.001	1.143 ± 0.001
10	2.893 ± 0.001	1.765 ± 0.001	3.791 ± 0.001	2.189 ± 0.001
20	3.102 ± 0.001	2.342 ± 0.001	4.182 ± 0.001	3.687 ± 0.001
30	3.343 ± 0.001	2.934 ± 0.001	5.203 ± 0.001	3.990 ± 0.001
40	3.948 ± 0.001	3.164 ± 0.001	5.542 ± 0.001	4.781 ± 0.001
50	4.530 ± 0.001	3.281 ± 0.001	5.789 ± 0.001	5.019 ± 0.001
60	4.903 ± 0.001	3.576 ± 0.001	6.783 ± 0.001	5.384 ± 0.001
70	5.492 ± 0.001	4.218 ± 0.001	7.293 ± 0.001	6.019 ± 0.001
80	5.920 ± 0.001	4.648 ± 0.001	7.734 ± 0.001	6.663 ± 0.001
90	6.326 ± 0.001	5.673 ± 0.001	7.920 ± 0.001	6.931 ± 0.001
100	7.543 ± 0.001	5.923 ± 0.001	8.563 ± 0.001	7.019 ± 0.001
110	7.759 ± 0.001	6.165 ± 0.001	8.917 ± 0.001	7.394 ± 0.001
120	8.463 ± 0.001	6.620 ± 0.001	9.645 ± 0.001	7.930 ± 0.001
130	8.742 ± 0.001	6.829 ± 0.001	10.647 ± 0.001	8.395 ± 0.001
140	9.432 ± 0.001	7.293 ± 0.001	11.367 ± 0.001	9.568 ± 0.001
150	9.921 ± 0.001	7.634 ± 0.001	11.928 ± 0.001	9.892 ± 0.001
160	9.994 ± 0.001	7.895 ± 0.001	12.893 ± 0.001	10.569 ± 0.001
170	10.289 ± 0.001	8.531 ± 0.001	12.994 ± 0.001	11.203 ± 0.001
180	11.293 ± 0.001	9.421 ± 0.001	13.563 ± 0.001	11.465 ± 0.001
190	11.563 ± 0.001	9.563 ± 0.001	14.092 ± 0.001	11.930 ± 0.001
200	12.082 ± 0.001	10.346 ± 0.001	14.672 ± 0.001	12.674 ± 0.001

0.3 ml digestion buffer was used. Samples were incubated for 18 h at 50 °C. Mixture of Phenol/Chloroform/Iso-amyl alcohol (25:24:1) was added to the samples in the same volume of the sample and then centrifuged at 15,000 rpm for 10 min. The top layer (aqueous) was transferred to a new tube. Then add half of the volume of the transferred aqueous layer 7.5 M $NH_4CH_3CO_2$ and add 100% ethanol in double volume of the transferred aqueous layer, then centrifuged for 2 min at 15,000 rpm. Samples were washed with 70% ethanol alcohol, left to dry in air and dissolved in 20 µL Tris-acetate EDTA buffer (pH 7.4) prior to electrophoresis using 1.2% agarose gel containing.

Statistical analysis

Extract analysis results were analysed by SPSS software (version 14). Data were expressed as mean ± SE. Comparison of mean values of studied variables between the two extracts was done using paired T test.

Table 4
Mean level of caspase-6 concentration (ng/ml) in both MCF-7 and AsPC-1 cells in response to treatment with dry and germinated fenugreek extracts.

Conc (μg/ml)	Dry seed extract		Germinated seed extract	
	MCF-7	AsPC-1	MCF-7	AsPC-1
control	3.213 ± 0.001	3.092 ± 0.001	3.213 ± 0.001	3.092 ± 0.001
10	5.093 ± 0.001	4.982 ± 0.001	6.394 ± 0.001	5.384 ± 0.001
20	7.023 ± 0.001	6.405 ± 0.001	7.804 ± 0.001	5.997 ± 0.001
30	7.495 ± 0.001	6.668 ± 0.001	8.017 ± 0.001	6.589 ± 0.001
40	9.304 ± 0.001	6.987 ± 0.001	9.923 ± 0.001	7.403 ± 0.001
50	9.984 ± 0.001	7.924 ± 0.001	10.378 ± 0.001	8.203 ± 0.001
60	10.384 ± 0.001	9.283 ± 0.001	12.034 ± 0.001	10.293 ± 0.001
70	11.374 ± 0.001	9.529 ± 0.001	13.983 ± 0.001	10.992 ± 0.001
80	13.583 ± 0.001	9.928 ± 0.001	16.394 ± 0.001	11.293 ± 0.001
90	14.239 ± 0.001	10.293 ± 0.001	17.934 ± 0.001	14.493 ± 0.001
100	15.394 ± 0.001	12.384 ± 0.001	19.304 ± 0.001	14.958 ± 0.001
110	15.495 ± 0.001	12.598 ± 0.001	19.982 ± 0.001	15.697 ± 0.001
120	16.034 ± 0.001	12.928 ± 0.001	21.093 ± 0.001	17.495 ± 0.001
130	17.394 ± 0.001	13.456 ± 0.001	21.394 ± 0.001	18.293 ± 0.001
140	17.674 ± 0.001	16.394 ± 0.001	21.875 ± 0.001	18.930 ± 0.001
150	17.992 ± 0.001	16.785 ± 0.001	22.394 ± 0.001	19.203 ± 0.001
160	18.029 ± 0.001	16.978 ± 0.001	22.903 ± 0.001	22.453 ± 0.001
170	18.384 ± 0.001	17.394 ± 0.001	23.918 ± 0.001	22.672 ± 0.001
180	18.734 ± 0.001	18.039 ± 0.001	25.013 ± 0.001	22.830 ± 0.001
190	20.395 ± 0.001	19.394 ± 0.001	25.198 ± 0.001	24.953 ± 0.001
200	20.484 ± 0.001	20.385 ± 0.001	30.475 ± 0.001	26.590 ± 0.001

Table 5
Effect of dry and germinated fenugreek extracts on LDH activity (U/ml) in both MCF-7 and AsPC-1 cells.

Conc (μg/ml)	Dry seed extract		Germinated seed extract	
	MCF-7	AsPC-1	MCF-7	AsPC-1
control	0.234 ± 0.001	0.198 ± 0.001	0.234 ± 0.001	0.198 ± 0.001
10	0.259 ± 0.001	0.204 ± 0.001	0.352 ± 0.001	0.253 ± 0.001
20	0.302 ± 0.001	0.219 ± 0.001	0.427 ± 0.001	0.304 ± 0.001
30	0.376 ± 0.001	0.258 ± 0.001	0.498 ± 0.001	0.339 ± 0.001
40	0.465 ± 0.001	0.298 ± 0.001	0.534 ± 0.001	0.429 ± 0.001
50	0.495 ± 0.001	0.320 ± 0.001	0.598 ± 0.001	0.461 ± 0.001
60	0.542 ± 0.001	0.353 ± 0.001	0.639 ± 0.001	0.492 ± 0.001
70	0.559 ± 0.001	0.421 ± 0.001	0.687 ± 0.001	0.537 ± 0.001
80	0.603 ± 0.001	0.465 ± 0.001	0.723 ± 0.001	0.583 ± 0.001
90	0.632 ± 0.001	0.497 ± 0.001	0.777 ± 0.001	0.621 ± 0.001
100	0.659 ± 0.001	0.502 ± 0.001	0.846 ± 0.001	0.663 ± 0.001
110	0.682 ± 0.001	0.559 ± 0.001	0.896 ± 0.001	0.692 ± 0.001
120	0.693 ± 0.001	0.592 ± 0.001	0.920 ± 0.001	0.728 ± 0.001
130	0.734 ± 0.001	0.611 ± 0.001	0.956 ± 0.001	0.769 ± 0.001
140	0.769 ± 0.001	0.637 ± 0.001	0.973 ± 0.001	0.834 ± 0.001
150	0.804 ± 0.001	0.663 ± 0.001	1.293 ± 0.001	0.857 ± 0.001
160	0.853 ± 0.001	0.684 ± 0.001	1.478 ± 0.001	0.921 ± 0.001
170	0.876 ± 0.001	0.754 ± 0.001	1.503 ± 0.001	0.967 ± 0.001
180	0.945 ± 0.001	0.816 ± 0.001	1.593 ± 0.001	0.992 ± 0.001
190	0.996 ± 0.001	0.859 ± 0.001	1.693 ± 0.001	1.238 ± 0.001
200	1.129 ± 0.001	0.903 ± 0.001	1.735 ± 0.001	1.428 ± 0.001

All biochemical results were also analysed by SPSS software (version 14). Data were expressed as mean ± SD. Comparison of mean values of studied variables among different groups was done using ANOVA test. $P < 0.05$ was considered to be significant (Levesque, 2007).

3. Results

3.1. Antioxidant characteristics

Our results showed that germination significantly increased all the antioxidant characters in germinated fenugreek seeds extract compared to that in the dry one. Total antioxidant capacity in germinated seeds extract increased about 172.9% more than that in the dry seed extract. Total reducing in germinated seeds extract increased about 82% more than that in the dry seed extract. Antioxidant enzymes (SOD, POX, CAT and PPO) activity in germinated seeds extract increased about 137.8, 123.1, 133.8 and 118.2% respectively more than that in the dry seed extract (Table 1).

3.2. Phytochemical components

Our study revealed that germination significantly increased all the studied phytochemical components in germinated fenugreek seeds extract than that in the dry one. The increase ratios in total alkaloids, total flavonoid, total phenolic, total steroids, Total saponin, Trigonelline content, Total tannins, Glycosidic Cyanide and Phytat were 68.8, 70.1, 80.7, 45.2, 120, 90.6, 173.2, 78.5 and 84.1 respectively (Table 2).

3.3. Effect of dry and germinated fenugreek extracts on cell viability of breast and pancreatic cancer cell lines

3.3.1. MCF-7 human breast cell line

Germinated seed extract reduced viability of MCF-7 cell line higher than dry seed fenugreek extract did. Dry seed extract showed significant inhibitory effect on MCF-7 human cell line after treatment with 50–100 μg/ml ($p < 0.05$) and 110–200 μg/ml ($p < 0.01$) for 24 h. Germinated seed extract showed highly significant inhibitory effect on this cell lines after treatment with 10–200 μg/ml ($p < 0.001$). The IC50 dose was between 100 and 110 μg/ml & 40 and 50 μg/ml for dry and germinated seed extracts respectively (Fig. 1).

3.3.2. AsPC-1 human breast cell line

Germinated seed extract reduced viability of AsPC-1 cell line higher than dry seed fenugreek extract did. Dry and germinated seed extract showed significant inhibitory effect on AsPC-1 human cell lines after treatment with 10–200 μg/ml ($p < 0.001$) for 24 h (Fig. 1). The IC50 dose was between 130 and 140 μg/ml and 90 and 100 μg/ml for dry and germinated seed extracts respectively.

3.4. Effect on caspase-3 and caspase-6 activity on both MCF-7 and AsPC-1 cell lines

Caspase-3 and caspase-6 activity was significantly increased after MCF-7 and AsPC-1 cells were treated with dry and germinated fenugreek extracts (10–200 μg/ml) as compared to the control group

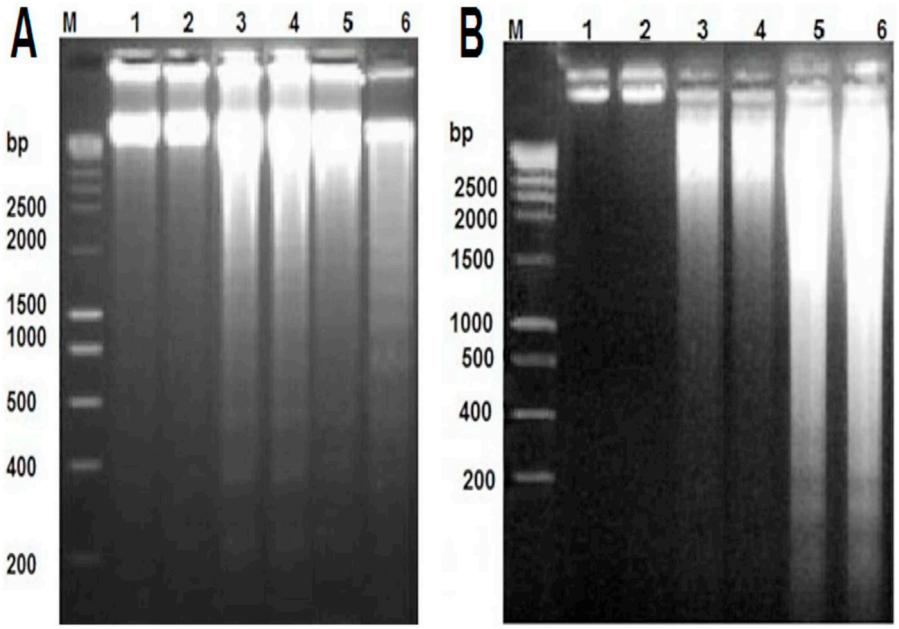

Fig. 2. DNA fragmentation induction in MCF-7 and AsPC-1 cell line. The DNA fragmentation analysis in MCF-7 (A) and AsPC-1 (B) cell lines after treatment with dry and germinated fenugreek extracts for 24 h. Lane M: Marker, lane. 1: untreated cells, lane. 2: 50 µg/ml dry extract in (A) and 90 µg/ml dry extract in (B), lane. 3: IC 50 of the dry extract, lane 4: IC 50 of the germinated extract, lane 5: dry extract in a concentration of 200 µg/ml, lane 6: germinated extract in a concentration of 200 µg/ml.

($P < 0.001$) (Tables 3 and 4). Germinated seed extract induced caspase-3 and -6 higher productions than dry seed extract did, in both cell lines. Also, caspase-3 and-6 concentration in cell line increased more than in AsPC-1 cells. The highest increase ratio in the caspase-3 (about 1084.2%) was found in MCF-7 cells under treatment with 200 mg/ml germinated seeds extract, while the lowest increase ratio in the caspase-3 (about 54.4%) was found in AsPC-1celld under treatment with 10 mg/ml dry seeds extract.

3.5. Effect on LDH activity on both MCF-7 and AsPC-1 cell lines

Dry and germinated fenugreek extracts caused significant ($P < 0.001$) membrane damage with the leakage of LDH enzyme. Cell damage was stronger with germinated than with dry fenugreek extracts; and was more intense in MCF-7 cells than I AsPC-1 (Table 5).

3.6. Induction of DNA fragmentation

One of the biochemical features of apoptosis is the fragmentation of genomic DNA. Fenugreek extracts were found to induce inter-nucleosomal degradation of DNA in all treatment concentrations, resulting in ladder-shaped nucleosomal DNA fragments compared to mock as shown in (Fig. 2).

4. Discussion

New effective and safe therapeutic agents for cancer are highly demanded. Many plant extracts can be used as therapeutic agents due to for their chemoprotective potentials (Seyed, 2019) Results revealed that germinated seed extract reduced viability of MCF-7 & AsPC-1 cell line higher than dry seed fenugreek extract. Indicating anti-proliferation activity of dry and germinated seed extract on MCF-7 & AsPC-1 cell line and raising the possibility that fenugreek extracts might be a potential chemo-preventive or therapeutic agent.

The therapeutic effects of natural extract is ascribed to their rich contents of phytochemicals components such as alkaloids, flavonoids, phenols and saponins (Murlidhar and Goswami, 2012; Pham et al., 2018). Germinated seeds extract has higher content of alkaloids, flavonoids, Phenols and saponins than that in the dry one, so it has higher anticancer activity than the dry seeds extract. Germinated seeds extract had also higher trigonelline and phytat content than the dry one. Trigonelline is an important alkaloid which increases the susceptibility of cancer cell line to apoptosis (Arlt et al., 2013). Trigonelline reported to have anticancer and antidiabetic activities (Jyothi et al., 2017). Phytate protective role in carcinogenesis is attributed to increasing in cell apoptosis (Jenab and Thompson, 2000), and this fact is clearly in our results as germinated seed extract has higher phytate content than that in the dry one, consequently the germinated seeds extract caused higher cell apoptosis than the dry seeds extract.

Many reports proved that the anticancer activity of certain extracts is highly correlated to their antioxidant characters (Alaklabi et al., 2017; Yen et al., 2017; Abdul Halim et al., 2018). This relationship between the antioxidant and anticancer activity was well demonstrated in our study: geminated seeds extract was more effective as anticancer agent than the dry one and also had higher antioxidant capacity.

Apoptosis can be induced in cells by different mechanisms. Death receptor and mitochondrial apoptotic pathways are the two major pathways that have been identified in mammalian cells. Mitochondria have a central role in activation of caspase cascade and apoptosis. Shafi et al. (2009) reported activation of procaspase-9 and then caspase-3 as a result of release of cytochrome-c from mitochondria. Increase in caspase-3 and caspase-6 activity was found in MCF-7 and AsPC-1 treated cells with dry and germinated fenugreek extracts, indicating that the anti-cancer activity of fenugreek extract was due to apoptosis induction. This is similar to the finding of Sebastian and Thampan (2007), Alshatwi et al. (2017) and Khalil et al. (2015) which revealed that treatment with fenugreek extract has a cytotoxic effect and apoptosis induction takes place in a dose-dependent manner through upregulation of caspase-3.

Raju et al. (2004) reported that Trigonella extract induced apoptosis in HT-29 human colon cancer cells due to its high saponins content. This explained the higher activity of caspases in the cells treated with the germinated seeds extract than that in the cells treated with the dry seeds extract, because germinated seeds extract had higher saponin content than the dry one.

Lactate dehydrogenase (LDH) is an enzyme found in cytoplasm of each cell. Membrane was damage cause its release into the extracellular medium. LDH activity is considered to be apoptosis marker. Dry and germinated fenugreek extracts caused significant membrane damage with the leakage of LDH enzyme. Our results are in line with Elshawy et al. (2016) who declared that silver nanoparticles caused significant increase of the lactate dehydrogenase (LDH) and caspase-3 leading to

induction of apoptosis in MCF-7 and MCT cancer cell lines which was further confirmed through increasing nuclear DNA damage.

Dry and germinated fenugreek extracts were found to induce internucleosomal degradation of DNA in all treatment concentrations. Trigonella extract caused degradation in the DNA due to its ability to interact with DNA topoisomerases resulting in inhibition of the DNA relaxation which is the molecular target for many antitumor drugs. This comes in the same line with Sebastian and Thampan (2007) who observed that the ethanolic fenugreek extract and soyabean extract reduced viability and caused early apoptotic changes in MCF-7 cells. Further, DNA fragmentation nearly 180–200 base pair has also been observed.

The higher apoptosis due to treatment with the germinated fenugreek extract can be related to its high content of trigonelline (90.64% more than dry one). This is correlated with Arlt et al. (2013) who reported that the alkaloid trigonelline renders pancreatic cancer cells more susceptible to apoptosis through decreased proteasomal gene expression and proteasome activity.

Conclusion

Both dry and germinated fenugreek extract induced cell death of both MCF-7 and AsPC-1 cell lines. Also, induced apoptosis by increasing caspase-3 and caspase-6 activity and DNA fragmentation. Germinated extract has higher antioxidant characters and phytochemical components content specially the alkaloids, saponins, trigonellin and phytat than that in the dry seeds extract. Therefore germinated seeds extract was more effective as anticancer than the dry seeds extract.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101067.

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