



## RESEARCH ARTICLE

## Cytotoxic Effects of *Pistacia khinjuk* Seed Extracts on Different Cell Lines and its Mitogenic Effects on Blood Lymphocyte *In Vitro*

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### ABSTRACT

Reports indicated that extract *Pistacia khinjuk* has anti-inflammatory, antipyretic, antibacterial, and antiviral, in treating of diarrhea and throat infections and has hepatoprotective effects against acetaminophen and carbon tetrachloride. This study was undertaken to investigate the possible cytotoxic effects of methanolic and aqueous seeds extract of *P. khinjuk* on different tumors (rhabdomyosarcoma [RD] and murine mammary adenocarcinoma [Ahmed-Mohammed-Nahi-2003 (AMN-3)]) and normal cell lines (murine fibroblast) and its mitogenic effects on blood lymphocytes. The cytotoxic effects of *P. khinjuk* seed extracts were evaluated on two tumor cell lines, RD and murine mammary adenocarcinoma (AMN-3) and one normal cell line, murine fibroblast (L20B). Moreover, the mitogenic effects of the plant extract were studied, on human blood lymphocytes. Both methanolic and aqueous seed extracts of *P. khinjuk* significantly induced tumor cell lines and the normal cell line proliferation, especially in highest concentrations. The results show that the extracts induced significant increases in human blood lymphocyte proliferation at 72 h. This activity of plant extracts recommends it as a good mitogenic agent in researches; in conclusion, seed extracts of *P. khinjuk* induced proliferation of all tested cell lines. High concentrations of both aqueous and methanolic seed extracts of *P. khinjuk* showed mitogenic effects.

**Keywords:** Mitogenic effect, *Pistacia khinjuk*, proliferative effect, tissue culture

### INTRODUCTION

Conventionally, *Pistacia khinjuk* used as tonic and expectorant, it has been used in cough, fever, and asthma.<sup>[1]</sup> Few investigations have been reported the effect of different crude and isolated compound extracts of *P. khinjuk* on different diseases. Some species of *Pistacia* have been used in folk medicine as anti-inflammatory, antipyretic, antibacterial, and antiviral, in treating diarrhea and throat infection.<sup>[2]</sup> Kordali *et al.* tested the antifungal activity of crude extracts obtained from the leaves of *Pistacia vera*, *Pistacia terebinthus*, and *Pistacia lentiscus*.<sup>[3]</sup> Ozcelik *et al.* used extracts of *P. vera* and studied antibacterial, antifungal, and antiviral properties of it, it showed little antibacterial activity, a noticeable antifungal activity, and significant antiviral activity.<sup>[4]</sup> Ljubuncic *et al.* studied the effects of aqueous extracts prepared from the leaves of *P. lentiscus* in experimental liver disease.<sup>[5]</sup>

Taran *et al.* (2009) studied the anthelmintic effect of *P. khinjuk* against protoscolecids of *Echinococcus granulosus*, the results of this study indicate *in vitro* anti-echinococcal activity of the essential oil of *P. khinjuk*.<sup>[6]</sup>

Derwich *et al.* (2010) studied antibacterial activity of the essential oil isolated from the leaf of *P. lentiscus*, the study was conducted to determine the phytochemistry and

antibacterial activities of *Pistacia* leaves oil against both Gram-negative and Gram-positive bacteria.<sup>[7]</sup> In another study, Taran *et al.* investigated the antimicrobial activity of the leaves of *P. khinjuk* and they found that some major constituents of essential oil from the aerial parts of *P. khinjuk* extracts showed antibacterial and antifungal activities.<sup>[1]</sup> Dizaye examined the effect of aqueous extract of *P. khinjuk* on acetaminophen and carbon tetrachloride-induced acute liver toxicity in albino rats, he concluded that an aqueous extract of *P. khinjuk* has hepatoprotective effects against acetaminophen and carbon tetrachloride.<sup>[8]</sup> Tohidi *et al.* investigated the antibacterial and wound healing activity in both *P. khinjuk* and *Pistacia atlantica*;

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the results suggested that antibacterial and wound healing activities of *P. khinjuk* are better than *P. atlantica* at the same concentrations.<sup>[2]</sup>

According to our knowledge, no data are available regarding the cytogenetic and mitogenic activities of *P. khinjuk*. Therefore, this study was designed to investigate the cytogenic effects of methanolic and aqueous seed extract of *P. khinjuk* on two tumor cell lines (rhabdomyosarcoma [RD] and murine mammary adenocarcinoma) and one normal cell line (murine fibroblast). Moreover, the mitogenic effects of *P. khinjuk* was studied on human blood lymphocytes.

## MATERIALS AND METHODS

### Plant Collection

*P. khinjuk* were collected from Erbil (Shaqalawa) Governorate. Plants were deposited to be identified, the identification done by the Department of Pharmacognosy, College of Pharmacy of Hawler Medical University, Iraq.

Seeds of *P. khinjuk* were separated and dried at room temperature according to Al-Barazanji. The dried seeds were grind into powder by electrical grinder (mesh no. 0.5 mm), and the powder was kept in plastic bags in a deep freezer ( $-20^{\circ}\text{C}$ ) until use.<sup>[9]</sup>

#### Preparation of aqueous extracts from *P. khinjuk* seeds

Aqueous extract was prepared from the grind powder according to Al-Barazanji. Then, crude dried extract was placed in labeled, tightly sealed plastic tubes, and stored at  $-20^{\circ}\text{C}$  until used.<sup>[9]</sup>

For the following experiments, 1 g of aqueous seeds extract was dissolved into 100 ml phosphate buffer saline (PBS), the suspension then filtered and sterilized using 0.2  $\mu\text{m}$  sterile Millipore filter and kept in a deep freeze in ( $-20^{\circ}\text{C}$ ) until use.

#### Preparation of methanolic extracts from *P. khinjuk* seeds

Methanolic extraction was prepared according to Harborn (1984), then the extract was stored in a tightly sealed plastic test tube at  $-20^{\circ}\text{C}$  until use.<sup>[10]</sup>

### Cell Lines

#### *Rhabdomyosarcoma (RD) cell line*

This is a human cell line derived from a biopsy specimen obtained from a pelvic rhabdomyosarcoma of a 7-year-old Caucasian girl.<sup>[11]</sup> Passages 258–263 of RD cell line were used throughout this study and RPMI-1640 was used for maintaining the cells.

#### *Ahmed-Mohammed-Nahi-2003 (AMN-3) cell line*

This cell line is a murine mammary adenocarcinoma cell line derived from a spontaneous mammary adenocarcinoma of female BALB/c mice.<sup>[12]</sup> The cells were maintained in RPMI-1640 medium. Passages 128–135 of AMN-3 cell line were used throughout this study.

#### *L20B cell line*

This cell line is a murine cell line derived from mouse L cells (fibroblasts) expressing the human poliovirus receptor.<sup>[13]</sup>

Passage numbers (14–18) of the L20B cell line were used in this study and it was maintained in RPMI-1640 medium containing 10% bovine calf serum.

### Cytotoxicity Assay

The cytotoxicity protocol was depending on Flick and Gifford, 1984.<sup>[14]</sup> Cells were plated in a 96-well flat-bottomed plate, after adhesion, serial dilutions of aqueous and methanolic extract separately three replicates for each concentration were placed in three columns for each type (200  $\mu\text{l}$  from each extract) to the appropriate wells and incubated for 24, 48, or 72 h at  $37^{\circ}\text{C}$ , 5–10%  $\text{CO}_2$  in a humidified environment. Untreated cells were used as controls. Then, the supernatants were decanted off and cells in each well were washed with PBS twice from, and 50  $\mu\text{l}$  of 0.01% neutral red dye was added to each well and reincubated for 2 h; at the end of incubation, excess dye was removed by washing the wells twice with 150  $\mu\text{l}$  PBS, then 125  $\mu\text{l}$  of extraction dye solution was added.<sup>[10]</sup> The optical density (OD) of each well was read using an enzyme-linked immunosorbent assay reader at a transmitting wavelength on 492 nm.

### Mitogenic Activity of *P. khinjuk* Seeds Extracts *In Vitro*

Human blood culture was prepared by collecting blood sample by vein puncture and transferred into heparinized tubes containing media (RPMI-1640); then, different extracts of *P. khinjuk* were added to blood culture tubes (three tubes for each) instate of polyhydroxyalkanoates (PHA) and three tubes prepared with PHA as control positive and other three without PHA as negative control. All tubes incubate in  $\text{CO}_2$  incubator and shaken gently each 15 min at the 1<sup>st</sup> h, 30 min at the 2<sup>nd</sup> h, and ones at the 3<sup>rd</sup> and 4<sup>th</sup> h then each 12 h and incubated for 48 and 72 h; then, the cells were harvested, slides prepared and stained according to Suman et al., 2006.<sup>[15]</sup>

### Statistical Analysis

Significance level was ascertained by one-way analysis of variance, followed by Student-Newman-Keuls multiple tests. Results were expressed as the mean  $\pm$  standard error of the mean.  $P < 0.05$  was considered statistically significant. All statistical procedures were performed with SPSS software version 16.

## RESULTS

### Cytotoxic Effects of Aqueous and Methanolic Extract of *P. khinjuk* Seeds on RD Tumor Cell Line

Tables 1 and 2 show the effect of both aqueous and methanolic seed extracts of *P. khinjuk* on RD tumor cell line. The inhibitory effect was found in the proliferation of RD tumor cells when treated with 78.125, 312.5, 625, and 1250  $\mu\text{g}/\text{ml}$  of seed aqueous extract for 24 h. However, an increase in the proliferation of RD cells was detected when these cells treated for 24 h with 10,000  $\mu\text{g}/\text{ml}$ , in which the OD was  $0.4773 \pm 0.0115$ . This induction effect was also detected at 48 h treatment with the same plant extract, in which a significant increase was observed at

**Table 1:** Cytotoxic effect of aqueous extract of *P khinjuk* seeds on the growth of RD tumor cell line

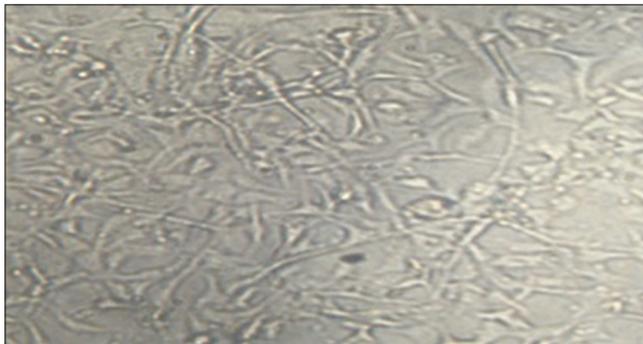
Concentrations (µg/ml)	Exposure period		
	24 h	48 h	72 h
Control	0.3987±0.008 <sup>b</sup>	0.1133±0.0003 <sup>a</sup>	0.1127±0.0019 <sup>a</sup>
78.125	0.3567±0.024 <sup>a</sup>	0.1413±0.0032 <sup>b</sup>	0.117±0.0051 <sup>a</sup>
156.25	0.3520±0.0144 <sup>a</sup>	0.1477±0.0067 <sup>bc</sup>	0.1097±0.0082 <sup>a</sup>
312.5	0.3440±0.0016 <sup>a</sup>	0.1467±0.0027 <sup>bc</sup>	0.1157±0.0113 <sup>a</sup>
625	0.3467±0.0043 <sup>a</sup>	0.1467±0.0027 <sup>bc</sup>	0.1233±0.0035 <sup>a</sup>
1250	0.3463±0.0018 <sup>a</sup>	0.1637±0.0059 <sup>bc</sup>	0.1293±0.0027 <sup>a</sup>
2500	0.3997±0.0023 <sup>b</sup>	0.1667±0.0023 <sup>c</sup>	0.132±0.0044 <sup>a</sup>
5000	0.3977±0.0009 <sup>b</sup>	0.1413±0.0032 <sup>c</sup>	0.1277±0.0062 <sup>a</sup>
10,000	0.4773±0.0115 <sup>c</sup>	0.1687±0.0064 <sup>c</sup>	0.1193±0.0049 <sup>a</sup>

*P khinjuk*: *Pistacia khinjuk*, RD: Rhabdomyosarcoma. \*Similar letters indicate no significant differences. \*Different letters indicate significant differences at  $P < 0.05$

**Table 2:** Cytotoxic effect of methanolic seeds extract of *P khinjuk* seeds on the growth of RD tumor cell line

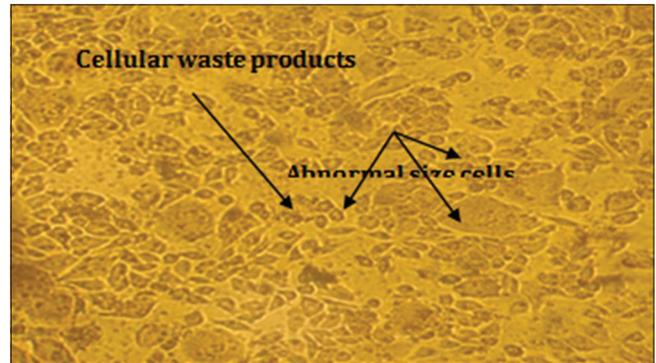
Concentrations(µg/ml)	Exposure period		
	24 h	48 h	72 h
Control	0.1327±0.0101 <sup>a</sup>	0.1470±0.0012 <sup>a</sup>	0.1427±0.004 <sup>ab</sup>
78.125	0.1547±0.0009 <sup>b</sup>	0.1543±0.0022 <sup>a</sup>	0.1704±0.0053 <sup>b</sup>
156.25	0.1637±0.0015 <sup>b</sup>	0.1543±0.0009 <sup>a</sup>	0.1627±0.0067 <sup>ab</sup>
312.5	0.147±0.0052 <sup>b</sup>	0.1540±0.0017 <sup>a</sup>	0.1667±0.0064 <sup>ab</sup>
625	0.1613±0.0018 <sup>b</sup>	0.1533±0.0035 <sup>a</sup>	0.1607±0.0046 <sup>ab</sup>
1250	0.1633±0.0047 <sup>b</sup>	0.1550±0.0038 <sup>a</sup>	0.1547±0.0015 <sup>ab</sup>
2500	0.1633±0.0019 <sup>b</sup>	0.1653±0.0018 <sup>b</sup>	0.1370±0.0131 <sup>a</sup>
5000	0.164±0.0027 <sup>b</sup>	0.1650±0.0012 <sup>b</sup>	0.1533±0.0026 <sup>ab</sup>
10,000	0.1667±0.0009 <sup>b</sup>	0.1690±0.0036 <sup>b</sup>	0.1537±0.0067 <sup>ab</sup>

*P khinjuk*: *Pistacia khinjuk*, RD: Rhabdomyosarcoma. \*Similar letters indicate no significant differences. \*Different letters indicate significant differences at  $P < 0.05$



**Figure 1:** Confluent monolayer of untreated rhabdomyosarcoma tumor cells (×400)

48 h of treatment, while the highest level of proliferation was detected in concentration of 10,000 µg/ml at 48 h of exposure with OD of 0.1687 ± 0.0064 when compared with its control (0.1133 ± 0.0003) [Figure 1]. In contrast, a non-significant difference was observed when this type of cell line was treated with aqueous seed extract of *P khinjuk* for 72 h in comparison with its control. Whereas the effect of methanolic extract of the same plant on RD tumor cell



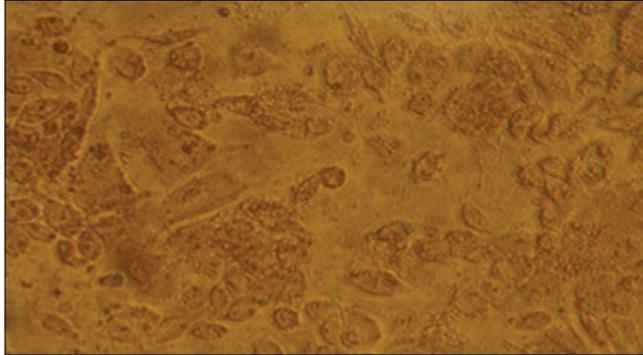
**Figure 2:** Rhabdomyosarcoma tumor cells treated with 10,000 µg/ml *Pistacia khinjuk* aqueous seeds extract 48 h (×400)

line was stimulation in growth of the cells at 24 h and 48 h of exposure, particularly at the higher concentrations (5000 µg/ml and 10,000 µg/ml), and the results showed non-significant differences at 72 h of treatment.

As shown in Figures 2 and 3, treatment of the cells with the highest concentrations of both extracts caused remarkable changes in the confluent monolayer appearance.

### Cytotoxic Effects of Aqueous and Methanolic Extract of *P. khinjuk* Seeds on AMN-3 Tumor Cell Line

The aqueous extract was more effective against the proliferation of AMN-3 cells at 24 h of treatment than



**Figure 3:** Rhabdomyosarcoma tumor cells treated with 10,000 µg/ml *Pistacia khinjuk* methanolic seeds extract 48 h (x400)

other treatment periods. The treatment for both 48 and 72 h showed non-significant differences in cell proliferation when compared with the control group, but the treatment for 24 h showed significant decreases in the proliferation of AMN-3 cells and the effect was started from 78.125 µg/ml to highest concentration of 10,000 µg/ml, Table 3.

Table 4 shows the effect of methanolic seeds extract of *P. khinjuk* on AMN-3 tumor cells which exhibited a significant increase in proliferation of tested cell line in all concentrations at all periods of treatment, particularly at the highest concentrations of 5000 µg/ml and 10,000 µg/ml.

Figure 4 shows the effect of aqueous seeds extract on AMN-3 cells at 24 h treatment, in which some cells with different changes in their shape and size were seen, the monolayer appearance is absent.

Figure 5 shows the shape and size of AMN-3 cells similar to that of untreated cells in control [Figure 6].

**Table 3:** Cytotoxic effect of aqueous extract of *P. khinjuk* seeds on the growth of AMN-3 tumor cell line

Concentrations(µg/ml)	Exposure period		
	24 h	48 h	72 h
Control	0.3190±0.2355 <sup>a</sup>	0.1030±0.0089 <sup>a</sup>	0.2267±0.0034 <sup>a</sup>
78.125	0.1027±0.0029 <sup>b</sup>	0.1080±0.0027 <sup>a</sup>	0.2097±0.0018 <sup>a</sup>
156.25	0.0963±0.0015 <sup>b</sup>	0.1067±0.0015 <sup>a</sup>	0.2233±0.0150 <sup>a</sup>
312.5	0.1067±0.0038 <sup>b</sup>	0.0960±0.0025 <sup>a</sup>	0.2303±0.0064 <sup>a</sup>
625	0.1060±0.0051 <sup>b</sup>	0.1070±0.0031 <sup>a</sup>	0.2594±0.0091 <sup>a</sup>
1250	0.1103±0.0060 <sup>b</sup>	0.1062±0.0029 <sup>a</sup>	0.2920±0.0089 <sup>a</sup>
2500	0.1160±0.0035 <sup>b</sup>	0.1340±0.0031 <sup>a</sup>	0.2910±0.0035 <sup>a</sup>
5000	0.1097±0.0019 <sup>b</sup>	0.1270±0.0038 <sup>a</sup>	0.2930±0.0076 <sup>a</sup>
10,000	0.1137±0.0023 <sup>b</sup>	0.1113±0.0033 <sup>a</sup>	0.2937±0.0033 <sup>a</sup>

\*Similar letters indicate no significant differences. \*Different letters indicate significant differences at  $P < 0.05$ . *P. khinjuk*: *Pistacia khinjuk*, AMN-3: Ahmed-Mohammed-Nahi-2003

**Table 4:** Cytotoxic effect of methanolic extract *P. khinjuk* seeds on the growth of AMN-3 tumor cell line

Concentrations (µg/ml)	Exposure period		
	24 h	48 h	72 h
Control	0.0820±0.0025 <sup>a</sup>	0.1030±0.0089 <sup>a</sup>	0.2267±0.0034 <sup>a</sup>
78.125	0.1007±0.0029 <sup>b</sup>	0.1133±0.0026 <sup>ab</sup>	0.2277±0.0069 <sup>a</sup>
156.25	0.0957±0.0020 <sup>b</sup>	0.1210±0.0012 <sup>abc</sup>	0.2307±0.0113 <sup>ab</sup>
312.5	0.1023±0.0038 <sup>b</sup>	0.1217±0.0020 <sup>bc</sup>	0.2410±0.0164 <sup>abc</sup>
625	0.1027±0.0033 <sup>b</sup>	0.1207±0.0035 <sup>bc</sup>	0.2443±0.0085 <sup>abc</sup>
1250	0.1020±0.0015 <sup>b</sup>	0.1347±0.0037 <sup>c</sup>	0.2823±0.0033 <sup>abc</sup>
2500	0.0997±0.0012 <sup>cd</sup>	0.1540±0.0049 <sup>e</sup>	0.2990±0.0006 <sup>abc</sup>
5000	0.1093±0.0018 <sup>d</sup>	0.1367±0.0024 <sup>d</sup>	0.3040±0.0025 <sup>bc</sup>
10,000	0.1103±0.0015 <sup>c</sup>	0.1160±0.0065 <sup>c</sup>	0.3093±0.0064 <sup>c</sup>

*P. khinjuk*: *Pistacia khinjuk*, AMN-3: Ahmed-Mohammed-Nahi-2003. \*Similar letters indicate no significant differences. \*Different letters indicate significant differences at  $P < 0.05$

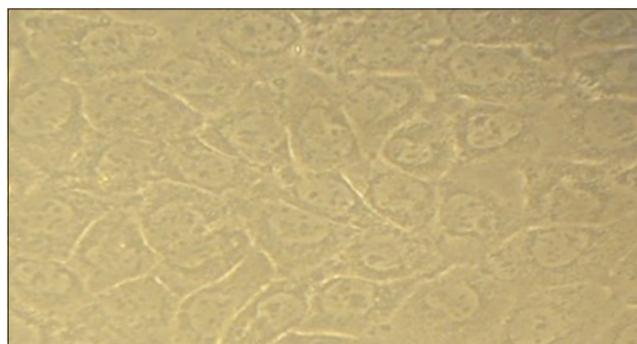
**Table 5:** Cytotoxic effect of aqueous extract of *P. khinjuk* seeds on the growth of L20B cell line

Concentrations ( $\mu\text{g/ml}$ )	Exposure period		
	24 h	48 h	72 h
Control	$0.0947 \pm 0.0013^a$	$0.1 \pm 0.0061^a$	$0.0993 \pm 0.0003^d$
78.125	$0.0940 \pm 0.0027^a$	$0.1 \pm 0.0015^a$	$0.0707 \pm 0.0012^a$
156.25	$0.0947 \pm 0.0032^a$	$0.1003 \pm 0.0009^a$	$0.0777 \pm 0.0007^b$
312.5	$0.0957 \pm 0.0039^a$	$0.1030 \pm 0.0006^a$	$0.0797 \pm 0.0007^b$
625	$0.960 \pm 0.0031^a$	$0.1030 \pm 0.0006^a$	$0.003 \pm 0.0019^b$
1250	$0.0963 \pm 0.0017^a$	$0.1077 \pm 0.0032^a$	$0.0777 \pm 0.0019^b$
2500	$0.0940 \pm 0.0015^a$	$0.1083 \pm 0.0015^a$	$0.0887 \pm 0.0032^c$
5000	$0.1140 \pm 0.0135^a$	$0.113 \pm 0.0007^a$	$0.1030 \pm 0.0025^d$
10,000	$0.1153 \pm 0.0019^a$	$0.1753 \pm 0.0058^b$	$0.0910 \pm 0.0021^c$

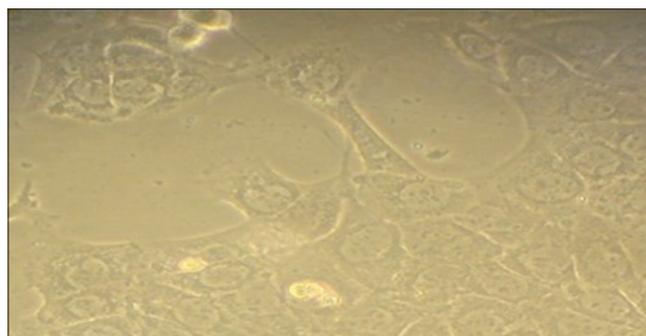
\*Similar letters indicate no significant differences.\*Different letters indicate significant differences at  $P < 0.05$ . *P. khinjuk*: *Pistacia khinjuk*,



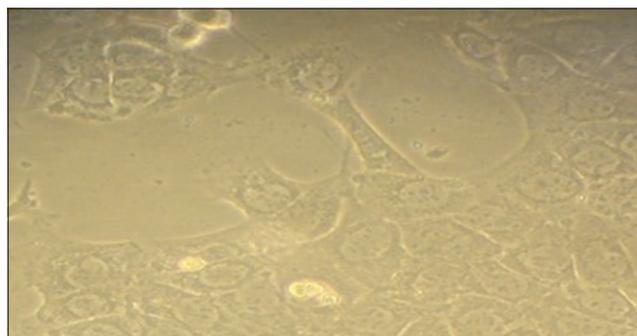
**Figure 4:** Ahmed-Mohammed-Nahi-2003 tumor cells treated with 10,000  $\mu\text{g/ml}$  of *Pistacia khinjuk* aqueous seeds extract at 24 h of exposure ( $\times 400$ )



**Figure 6:** Confluent monolayer of untreated Ahmed-Mohammed-Nahi-2003 tumor cells ( $\times 400$ )



**Figure 5:** Ahmed-Mohammed-Nahi-2003 tumor cells treated with 5000  $\mu\text{g/ml}$  of *Pistacia khinjuk* methanolic seeds extract at 72 h of exposure ( $\times 400$ )



**Figure 7:** Confluent monolayer of untreated L20B cells ( $\times 400$ )

### Cytotoxic Effects of Aqueous and Methanolic Extract of *P. khinjuk* Seeds on L20B Cell Line

Table 5 shows the effect of *P. khinjuk* aqueous seeds extract on the growth of L20B cells. During the first 2 days of incubation, the aqueous extract did not stimulate the growth of L20B cells as compared with control [Figure 7].

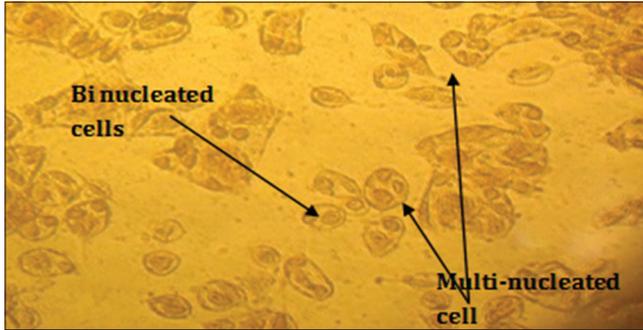
Only a significant induction of cell proliferation was detected when treated with 10,000  $\mu\text{g/ml}$  for 48 h of exposure. After 72 h of treatment, the aqueous extract significantly

inhibited cell growth at almost all concentrations. This effect is shown in Figure 8, in which the cells exhibited different changes in cell shape. Some rounded, binucleated, and multinucleated cells were detected when treated with 10,000  $\mu\text{g/ml}$ .

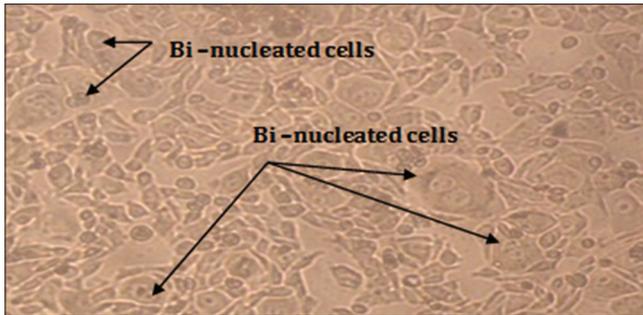
Viability of L20B cells after treatment with the methanolic extract is shown in Table 6. Treatment of cells with concentrations of 2500  $\mu\text{g/ml}$  and 10,000  $\mu\text{g/ml}$  for 24 h caused a significant increase in cell viability. All the other concentrations have no significant effects on cell viability. Non significant differences were detected when the cells treated with 78.125, 156.25, 312.5, and 625  $\mu\text{g/ml}$  at 48 h, while the cells when treated with higher concentrations 1250, 2500, 5000, and 10,000  $\mu\text{g/ml}$  at 48 h a significant increases were

detected in cell viability. The results also showed insignificant differences at 72 h of exposure.

Figure 9 shows the induction effect of this extract 10,000 µg/ml for 48 h on L20B cells with different changes such as rounded and binucleated and multinucleated cells.



**Figure 8:** L20B cells treated with 10,000 µg/ml of *Pistacia khinjuk* aqueous seeds extract at 72 h of exposure (×400)



**Figure 9:** L20B cells treated with 10,000 µg/ml of *Pistacia khinjuk* methanolic seeds extract at 48 h (×400)

### Mitogenic Activity of Aqueous and Methanolic Extracts of *P. khinjuk* on Human Blood Lymphocyte

Aqueous and methanolic extracts of seeds of *P. khinjuk* 10,000 µg/ml were used to detect its effect as mitogen in blood lymphocyte cultures for both 48 and 72 h exposure; the results were compared with positive control group which was treated with PHA as a mitogen and also were compared with the negative control group (group that treated with PBS).

Significant induction was detected in all treated group with 10,000 µg/ml *P. khinjuk* seeds extract for 48 h when compared with negative control group, and non significant differences was detected when the cells treated with aqueous and methanolic extracts for 72 h in all groups when compared with positive control group, but a significant induction was recorded in Mitotic Index (MI) of all groups when compared with the negative control group, Table 7.

### DISCUSSION

#### Effects of Aqueous and Methanolic Seeds Extracts of *P. khinjuk* on RD, AMN-3, and L20B Cell Lines

When both cell lines (RD and AMN-3) treated with seed extract of *P. khinjuk*, a significant increases in cell proliferation were detected for both aqueous and methanolic extracts, except exposure of both types of cell lines for 72h to aqueous extract, this increase might be due to the fact that extractions from some herbs can restrain cancer cells and induce its proliferation. Cao *et al.* estimated that extractions from some herbs can restrain hepatocarcinoma, and the herbal extractions of flavonoid and arsenic trioxide cannot only

**Table 6:** Cytotoxic effect of methanolic extract of *P. khinjuk* seeds on the growth of L20B cell line

Concentrations (µg/ml)	Exposure period		
	24 h	48 h	72 h
Control	0.1±0.0061 <sup>a</sup>	0.1001±0.0061 <sup>a</sup>	0.993±0.0003 <sup>ab</sup>
78.125	0.1±0.0012 <sup>a</sup>	0.1003±0.0067 <sup>a</sup>	0.0913±0.0017 <sup>a</sup>
156.25	0.1003±0.0019 <sup>a</sup>	0.1003±0.0049 <sup>a</sup>	0.0920±0.0006 <sup>a</sup>
312.5	0.1127±0.0029 <sup>a</sup>	0.1027±0.0024 <sup>a</sup>	0.0937±0.0026 <sup>a</sup>
625	0.1113±0.0009 <sup>a</sup>	0.1323±0.0024 <sup>a</sup>	0.1037±0.0047 <sup>ab</sup>
1250	0.1270±0.0050 <sup>a</sup>	0.2417±0.0197 <sup>d</sup>	0.1033±0.0018 <sup>ab</sup>
2500	0.1777±0.0112 <sup>c</sup>	0.2430±0.0103 <sup>bd</sup>	0.1120±0.0027 <sup>b</sup>
5000	0.1317±0.0038 <sup>a</sup>	0.2073±0.0049 <sup>d</sup>	0.0910±0.0012 <sup>b</sup>
10,000	0.1550±0.1617 <sup>b</sup>	0.2977±0.0089 <sup>e</sup>	0.1030±0.0060 <sup>ab</sup>

\*Similar letters indicate no significant differences. \*Different letters indicate significant differences at P<0.05. *P. khinjuk*: *Pistacia khinjuk*

**Table 7:** Mean±SEM for mitotic index of human blood lymphocyte when *P. khinjuk* used as mitogenic factor

Exposure period	Positive control (PHA)	Negative control (PBS)	<i>P. khinjuk</i> methanolic seeds extract 10,000 µg/ml	<i>P. khinjuk</i> aqueous seeds extract 10,000 µg/ml
48 h treatment	0.4893±0.00445 <sup>a</sup>	0.2438±0.00185 <sup>d</sup>	0.3564±0.02775 <sup>c</sup>	0.3767±0.00845 <sup>c</sup>
72 h treatment	0.4906±0.00561 <sup>a</sup>	0.2862±0.29093 <sup>b</sup>	0.3822±0.00740 <sup>a</sup>	0.3807±0.00491 <sup>a</sup>

\*Similar letters indicate no significant differences. \*Different letters indicate significant differences at P<0.05. *P. khinjuk*: *Pistacia khinjuk*, PHA: Polyhydroxyalkanoates, PBS: Phosphate buffer saline, SEM: Standard error of the mean

restrain the proliferation of hepatocarcinoma cells but also induce apoptosis of hepatocarcinoma cells.<sup>[16]</sup>

Rui-Chuan *et al.* reported that Chinese medicine can induce cytodifferentiation of hepatocarcinoma, also rhubarb acid could restrain cell proliferation of various tumors such as mastocarcinoma, lung cancer, hepatocarcinoma, and colon carcinoma, and has synergistic effects when used in combination with Mutamycin.<sup>[17]</sup> It could inhibit synthesis of DNA, promote apoptosis of cancer cells, and also it has been found that the inhibitory effect of repression of rhubarb on cancer cells is closely related with cell signal transduction.<sup>[18]</sup>

Tohidi *et al.* and Azadpour *et al.* found that the extract of *P. khinjuk* showed faster healing compared with control groups, faster wound contraction rate may be due to the presence of flavonoids, which is responsible for the free radical scavenging activity that is believed to be one of the most important components of wound healing that also be an evidence for the increased number of cells when treated with *P. khinjuk* extracts which may be caused rearrangement of the cell and induction in there proliferation by the presence of some of those phytochemical compounds such as flavonoids.<sup>[2,19]</sup>

In general, oleic and linoleic acids are known for their anti-inflammatory properties. Linoleic and alpha-linoleic acid provide lipids necessary for cell membrane repair and cellular respiration.<sup>[20]</sup>

Tavakoli and Pazhouhanmerhr found that *P. khinjuk* fruits when analyzed contain linoleic and linoleic acid, and the presence of these two types of essential fatty acids may be caused induction in cell proliferation when used for cell membrane repair.<sup>[21]</sup>

Moulos *et al.*, 2009, demonstrated that exposure of Lewis lung carcinomas to mastic oil (mastic oil from *P. lentiscus*) caused a time-dependent alteration in the expression of 925 genes.<sup>[22]</sup>

Mirian *et al.*, 2015, found that after the exposure of cell lines to methanolic extracts of three plants, the cells shows high survival when they treated with *P. khinjuk* extract in comparison with other two extracts, and this property may be due to the presence of essential oils.<sup>[23]</sup>

The effect of those extracts on L20B normal cells was also an induction of cell proliferation at high concentrations of all types of extracts and this induction in currently applied extract, lacks selectivity, and does not spare normal (non-malignant) cells from cancer cells, it also often induces resistance of tumor cells to antineoplastic agents, generally in the form of "multidrug resistance" to structurally unrelated compounds.<sup>[24]</sup>

### Mitogenic Activity of Aqueous and Methanolic Extracts of *P. khinjuk* Seeds on Human Blood Lymphocyte

Aqueous and methanolic extracts from both leaves and seeds of *P. khinjuk* were tested as a mitogen on blood lymphocyte. The results of the present study showed non-significant differences were found between the values of MI of each human lymphocyte that treated for 72 h either by aqueous or methanolic seeds extracts of *P. khinjuk* and positive control group (PHA), the same results were obtained after

the treatment with methanolic leaves extract for 48h. These results indicated that the activity of all these extracts is similar to that of PHA.

The increase in a number of cells when treated with *P. khinjuk* extracts may be caused by rearrangement of the cells and induction in their proliferation according to the presence of some phytochemical compounds such as flavonoids.<sup>[2]</sup>

Tavakoli and Pazhouhanmerhr found that *P. khinjuk* fruits when analyzed contain linoleic and linoleic acid and the presence of these two types of essential fatty acids may be caused induction in cell proliferation when used for repairing of the cell membrane.<sup>[21]</sup>

Ghosh and Gaba mentioned that *P. khinjuk* fruits and leaves have wound healing properties which may be due to the presence of different phytochemical compounds in these extracts.<sup>[25]</sup>

## CONCLUSIONS

Methanolic and aqueous seed extracts of *P. khinjuk* induced proliferation of all tested cell lines. High concentrations of both aqueous and methanolic seed extracts of *P. khinjuk* showed mitogenic effects.

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