



## Sensitivity of hematopoietic malignant cells to *Peganum harmala* seed extract *in vitro*

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### Abstract

**Background and Objective:** *Peganum harmala* is a medicinal plant that has been used for treatment of numerous diseases including viral, bacterial and parasitic infections. Anti-proliferative and anti-tumor effects of *Peganum harmala* extracts and its derivatives have been reported. In the present study, cytotoxic effect of *Peganum harmala* seeds aquaous extract on leukemic U937 and Molt-4 cells was evaluated *in vitro*.

**Materials and Methods:** U937 and Molt-4 cells were cultured in RPMI with 10% FBS. Then, the cells at logarithmic growth phase were incubated with different concentrations of aquaous extract of *Peganum harmala* seeds (0.1-5 mg/ml) for 24, 48 and 72 hours. Then, viability and proliferative response of leukemic cell lines was evaluated by trypan blue dye exclusion (TB) and MTT assays, respectively.

**Results:** *Peganum harmala* aquaous extract has a cytotoxic effect on leukemic cells used in this study, dose and time-dependently. This cytotoxicity was shown at  $\geq 0.5$  mg/ml after 24 hours and at  $\geq 0.1$  mg/ml after 48 and 72 hours incubation time. The *Peganum harmala* seeds aqueous extract cytotoxicity at  $> 0.2$  mg/ml concentration was significantly increased with time in this order: 72 h $>$ 48 h $>$ 24 h.

**Conclusion:** The leukemic cells used in this study showed sensitivity to *Peganum harmala* seeds aquaous extract dose and time dependently. This sensitivity significantly increased with time at  $> 0.2$  mg/ml concentration. *Peganum harmala* seeds aquaous extract shows cytotoxicity for leukemic cells and might be a valuable natural candidate in development of innovative therapeutic procedures for leukemia and probably other cancers.

**Key words:** *Peganum harmala*, Cytotoxicity, Leukemia

### 1. Introduction

**P***eganum harmala* (*P. harmala*) is a medicinal plant that has been used for treatment of numerous diseases such as viral, bacterial and parasitic infections (1-3). Many therapeutic effects of *P. harmala* including cytotoxicity, apoptotic and anti-tumoral activities have been ascribed to its ingredients, especially B-carboline (4, 5). Anti-proliferative effect of harmine (a beta carboline alkaloid derived from *P. harmala*) on a leukemic cell line has been reported (6). Furthermore, induction of apoptosis by harmine has been described (7). Also, the anti-inflammatory effect of harmine has been shown (8). Anti-angiogenic and anti-tumor properties of harmine have also been shown (9-11). Inhibition of tumor proliferation in gastric cancer by harmine-

paclitaxel complex has been demonstrated (12). Moreover, down regulation of tumor angiogenesis by a harmine derivative has also been revealed (13). Besides, anti proliferation and differentiation properties of *P. harmala* derivatives in leukemia cells has been determined (14). In addition, cytotoxicity of harmalidine (a novel indole alkaloid from *P. harmala*) against leukemia cells has been shown (15). The *P. harmala* extract exhibited cytotoxicity and reduced the growth of breast cancer cell lines (16, 17). A synthetic compound of carboline derivative (B-9-3) from *P. harmala* presented a significant anti-cancer effect against human lung, breast and colorectal carcinoma cell lines (5).

Inhibition of the ovarian cancer growth by harmine has also been verified (18). The suppression of cell

proliferation, tumor growth, invasion, and metastasis and matrix metalloproteinase-9 expression by harmine in non-small cell lung cancer (NSCLC) has been reported (19). Moreover, prevention of oxidative stress by *P. harmala* extract in Parkinson's disease in rats has been demonstrated (20). In the present study, the cytotoxic effect of *P. harmala* aqueous extract on leukemic U937 and Molt-4 cells was evaluated *in vitro*.

## 2. Materials and Methods

### 2.1. Reagents

*Peganum harmala* seeds were bought from local market. RPMI-1640 medium, penicillin, streptomycin, and trypan blue (TB) were purchased from Sigma (USA). Fetal calf serum (FCS) was obtained from Gibco (USA) and MTT (3-[4, 5-dimethyl thiazol-2, 5-diphenyltetrazoliumbromide]) kit was purchased from Invitrogen (USA). Microtiter plates, flasks and tubes were purchased from Nunc (Falcon, USA).

### 2.2. Peganum harmala seeds extract preparation

One hundred g of authenticated *P. harmala* dried seeds were chopped and boiled in 1 liter of distilled water. Then, solution was filtered and dried by vaporization. The dried extract was dissolved in RPMI-1640 and filtered by 0.2 µm filter and stored at -20°C until use in next experiments. The freeze-dried extract was diluted in culture medium to make the required concentrations before its use.

### 2.3. Cell lines

Human leukemic monocyte [U937 (NCBI C130)] and T cells [Molt-4 (NCBI C149)] were purchased from NCBI (National Cell Bank of Iran, Pasteur Institute of Iran, Tehran). The cells were conserved in RPMI-1640 medium supplemented with 10% FCS in 5% CO<sub>2</sub> at 37°C.

### 2.4. Cell culture and treatment

Leukemic cells were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin 100 IU/ml and streptomycin (100 µg/ml) at 37°C in 5% CO<sub>2</sub>. The cells were spread at a density of  $2 \times 10^4$  cell/well and then incubated with different concentrations of *P. harmala* seeds aqueous extract (0.1- 5 mg/ml) for 24, 48 and 72 hours. All experiments were done in triplicate.

### 2.5. Cell proliferation assay

The effect of different concentrations of *P. harmala* seeds aqueous extract on proliferation of the leukemic

cells was assessed by trypan blue dye exclusion (TB test) (21) and MTT assay (22).

### 2.6. Trypan blue dye exclusion assay

Base of trypan blue dye exclusion test is drop-out of dye by viable cells and taking it up by dead cells. Viability is assessed by direct calculating of viable and dead cells. Proportion of number of the viable cells to the whole number of cells is considered as viability ratio.

### 2.7. MTT assay

In MTT test the conversion of yellow water soluble MTT to a blue-insoluble formazon was evaluated according to the method established by Mosmann (22). At the end of incubation time, the medium was substituted with 100 µl of fresh medium. Then, 10 µl of MTT solution (5 mg/ml in PBS) was added to all wells and incubated at 37°C for 4 hours. Then, 100 µl of the SDS-HCl solution (100 mg SDS was dissolved in 1 ml of HCl) was added to each well and incubated at 37°C for 4 hours. Consequently, the insoluble formazon derivative was dissolved and absorbance was measured at 570 nm using a microplate reader (Biotek ELX 800). The results were indicated as cell numbers per control.

### 2.8. Statistical analysis

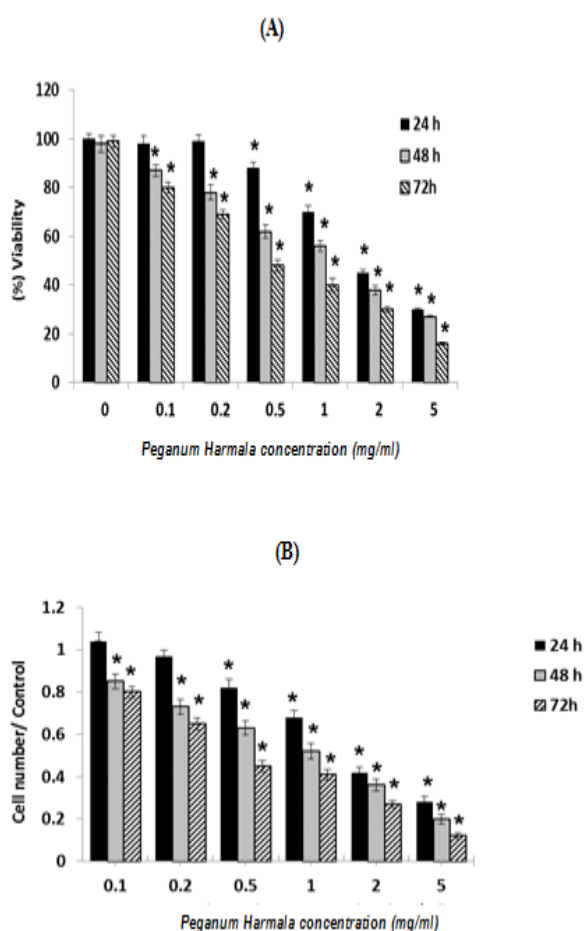
The effect of *P. harmala* seeds aqueous extract on each cell line was performed in three independent experiments (n = 3) and the results were expressed as mean ± SD. Statistical comparisons between groups were made by analysis of variance (ANOVA). P < 0.05 was considered significant. Tukey's test of multiple comparison was applied (5%) for statistically significant differences. For statistical analysis and graph drawing, the software SPSS 16.0 and Excel 2003 were used, Respectively.

## 3. Results

### 3.1. Toxicity of Peganum harmala seeds aqueous extract on U937 cells

Cytotoxic effect of different concentrations of *P. harmala* seeds aqueous extract on U937 cells at three time points are shown in Figures 1A and 1B. A and B show the results of trypan blue dye exclusion and MTT tests, respectively. *P. harmala* seeds aqueous extract significantly declined proliferation of U937 cells in both staining assays in all time intervals in a dose-dependent manner (p < 0.05) (Figures 1A and 1B). The results demonstrated in Figures 1A and 1B showed that *P. harmala* seeds aqueous extract significantly decreases the propagation of U937 cells at ≥ 0.5 mg/ml concentration after 24 hours incubation compared with untreated control cells (p < 0.05).

Also, the results revealed in Figures 1A and 1B showed that *P. harmala* seeds aqueous extract significantly decreases the proliferation of U937 cells at  $\geq 0.1$  mg/ml concentration after 48 and 72 hours incubation time in comparison with untreated control cells ( $p < 0.05$ ). Moreover, these results showed that there was not any significant difference in *P. harmala* seeds aqueous extract cytotoxicity between 48 and 72 hours incubation times at  $\leq 0.2$  mg/ml concentration. However, the *P. harmala* aqueous extract cytotoxicity at  $> 0.2$  mg/ml concentration significantly increased with time in this order: 72 h  $>$  48 h  $>$  24 h.

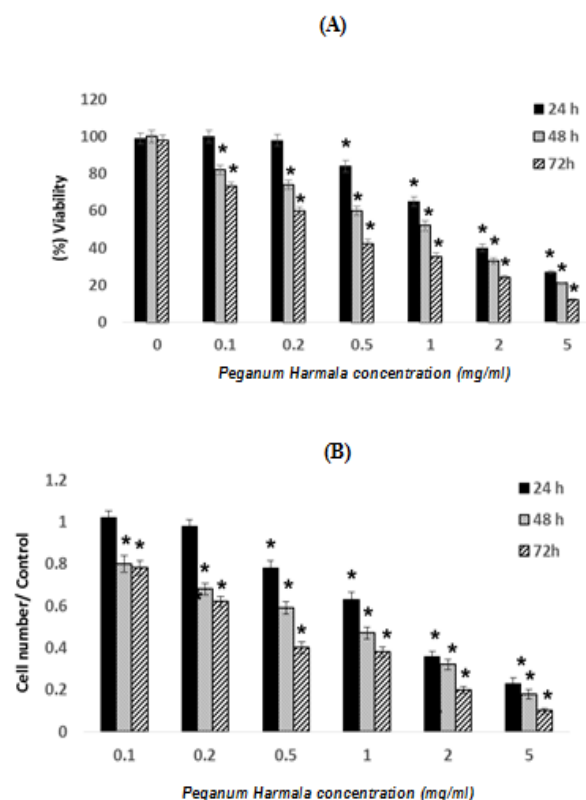


**Figure 1.** Cytotoxicity of *Peganum harmala* seeds aqueous extract on U937 cells. The U937 cells were treated with different concentrations of *Peganum harmala* seeds aqueous extract (0.1 to 5 mg/ml) for 24, 48 and 72 h. The results are presented as % of viability demonstrated by trypan blue dye exclusion (TB) test (A) and cell number/ control demonstrated by MTT assay (B). Data are mean  $\pm$  SEM of triplicate cultures.  $n = 3$ ;  $p < 0.05$  was considered significant.

### 3.2. Toxicity of *Peganum harmala* seeds aqueous extract on Molt-4 cells

The cytotoxicity of various concentrations of *P. harmala* seeds aqueous extract on Molt-4 cells at three

time points are shown in Figures 2A and 2B. A and B represent the data of trypan blue dye exclusion and MTT assays, respectively. *P. harmala* seeds aqueous extract significantly reduced proliferative responses of Molt-4 cells in both staining tests in all time points dose-dependently ( $p < 0.05$ ) (Figures 2A and 2B). The results exhibited in Figures 2A and 2B showed that *P. harmala* seeds aqueous extract significantly decreases the proliferation of Molt-4 cells at  $\geq 0.5$  mg/ml concentration after 24 hours incubation compared with untreated control cells ( $p < 0.05$ ). Besides, these data showed that *P. harmala* seeds aqueous extract significantly reduces the proliferative response of Molt-4 cells at  $\geq 0.1$  mg/ml concentration after 48 and 72 hours incubation time in comparison with untreated control cells ( $p < 0.05$ ). Moreover, these data indicated that there was no significant difference in *P. harmala* seeds aqueous extract toxicity among 48 and 72 hours incubation time at  $\leq 0.2$  mg/ml concentration. But, the *P. harmala* seeds aqueous extract cytotoxicity at  $> 0.2$  mg/ml concentration was significantly greater with time in this order: 72 h  $>$  48 h  $>$  24 h.



**Figure 2.** Cytotoxicity of *Peganum harmala* seeds aqueous extract on Molt-4 cells. The Molt-4 cells were treated with different concentrations of *Peganum harmala* seeds aqueous extract (0.1 to 5 mg/ml) for 24, 48 and 72 h. The results are presented as % of viability demonstrated by trypan blue dye exclusion (TB) test (A) and cell number/control demonstrated by MTT assay (B). Data are mean  $\pm$  SEM of triplicate cultures.  $n = 3$ ;  $p < 0.05$  was considered significant.

#### 4. Discussion

Natural substances could be useful in development of novel and effective treatments. According to the results of this study, the aqueous extract of *P. harmala* seeds has cytotoxic effects on both human monocytes and T leukemic cell lines. This cytotoxicity was dose and time dependent as shown at  $\geq 0.5$  mg/ml concentration of the extract at 24 h incubation time and at  $\geq 0.1$  mg/ml concentration of the extract at 48 and 72 h incubation time. The *P. harmala* seeds aqueous extract cytotoxicity at  $\geq 0.5$  mg/ml concentration was significantly greater with time in this order: 72 h > 48 h > 24 h. Accordingly, longer incubation time with *P. harmala* seeds aqueous extract increases its cytotoxicity in  $\geq 0.5$  mg/ml concentration. The pattern of *P. harmala* seeds cytotoxicity was similar for U937 and Molt-4 cells. Consistent to our findings, the anti-proliferative effect of hydroalcoholic extract of *P. harmala* seeds on human cervical carcinoma (HeLa) cells has been reported by Frouzandeh et al study (23). Similar to us in Frouzandeh et al study, hydroalcoholic extract of *P. harmala* seeds showed cytotoxicity in HeLa cells, dose- and time-dependently (23). However, Frouzandeh et al reported *P. harmala* seeds extract cytotoxicity on HeLa cells at  $\geq 12$   $\mu$ g/ml after 24 hours (23), while in our study *P. harmala* seeds extract did not show any cytotoxicity on monocytes and T leukemic cells at  $< 0.5$  mg/ml (500  $\mu$ g/ml) concentration of the extract after 24 h incubation time. The inconsistency between our results and Frouzandeh et al (23) may be due to different cell lines, type of extract, and used solvent. Frouzandeh et al used human cervix cancer HeLa cell line but we used human monocytic and T leukemia cells. Different cells have different sensitivities to drugs (24). Besides, we used aqueous extract of *P. harmala* seeds while Frouzandeh et al (23) utilized hydroalcoholic extract of *P. harmala* seeds. It is obvious that the hydroalcoholic extract ingredients are not as the same as that in aqueous extract. Moreover, Frouzandeh et al dissolved the dried hydroalcoholic extract of *P. harmala* seeds in dimethyl sulfoxide (DMSO) for preparing the different concentrations of the extract (23). The cytotoxicity of DMSO at  $\geq 2\%$  after 24 hours incubation time has been shown by us before (25). Consequently, in Frouzandeh et al study, the existence of DMSO used as a solvent might augment the cytotoxic effect of hydroalcoholic extract of *P. harmala* seeds.

The cytotoxicity and anti-inflammatory effects of *P. harmala* seed extract in human macrophages has been reported by Mahajn et al (26). In Mahajn et al study, *P. harmala* seed aqueous extract exerted cytotoxic effect on human THP1 macrophages at  $> 250$   $\mu$ g/ml in the presence of 1  $\mu$ g/ml lipopolysaccharide (LPS) and at 500  $\mu$ g/ml in the absence of LPS after 24 hours as was determined by lactate dehydrogenase (LDH) test

(26). This result of Mahajn et al study is consistent to us as in our study *P. harmala* seed aqueous extract showed cytotoxic effects on both human monocytes and T leukemic cell lines at  $\geq 0.5$  mg/ml (500  $\mu$ g/ml) concentration of the extract without LPS after 24 h incubation time. However, Mahajn et al used vitamin D3 and PMA-differentiated THP-1 cell line to macrophages were subsequently exposed to various concentrations of *P. harmala* seed extract (0-500  $\mu$ g/ml) for 24 h. But in our study we utilized undifferentiated human leukemic monocyte [U937] and T cells [Molt-4] exposed to different concentrations of *P. harmala* seed extract (0.1-5 mg/ml) for 24, 48 and 72 h. Anti-tumoral and anti-inflammatory effects of *P. harmala* extract or derivatives have been shown by several investigators (5-8, 16, 17). For example, the *P. harmala* extract exhibited cytotoxicity and reduced the growth in breast cancer cell lines (16, 17).

Many therapeutic effects of *P. harmala* including cytotoxicity, apoptosis induction and anti-tumoral activities have been ascribed to its ingredients, especially B-carboline (4, 5). In this regard, cytotoxicity of harmalidine (a novel indole alkaloid from *P. harmala*) against leukemia cells has been shown (15). A synthetic compound of carboline derivative (B-9-3) from *P. harmala* presented a significant anti-cancer effect against human lung, breast and colorectal carcinoma cell lines (5). Also, DH332, a synthetic  $\beta$ -carboline alkaloid from *P. harmala*, inhibited B cell lymphoma growth *in vitro* and *in vivo* (27). Moreover, down regulation of tumor angiogenesis by a harmine derivative has been reported (13). Furthermore, the anti-inflammatory properties of alkaloid extracts from seeds of *P. harmala* have been revealed (8, 28, 29). As macrophages and T cells play an essential role in inflammation (30), anti-inflammatory effects of *P. harmala* might be partly due to its cytotoxicity on inflammatory cells. Additionally, inflammation has a key role in pathology of leukemia (31) and beneficial role of anti-inflammatory compounds in leukemia has been reported (32). Since, the existing therapeutic medications for leukemia have not been very effective, *P. harmala* may be a possible promising candidate in preparation of new therapeutic medication for leukemia.

The results of the present study indicated that there are some hydrophilic toxic constituents in *P. harmala* for leukemic cells. In order to avoid its likely side effects, studies of *P. harmala* aqueous extract on normal cells are suggested. Moreover, further studies are required to define the sensitivity of different cells to *P. harmala* extract. In addition, further investigations are necessary to isolate and characterize the *P. harmala* aqueous extract constituents mediating cytotoxic effects as well as to delineate the molecular mechanism(s) of *P. harmala* aqueous extract cytotoxicity.



## Conclusion

*P. harmala* seeds aquaous extract shows cytotoxicity for leukemic cells and might be a valuable natural candidate in development of innovative therapeutic procedures for leukemia and probably other cancers.

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