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The Role of Harmine in Up-regulating p53 Gene Expression and Inducing Apoptosis in MCF-7 Cell Line

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Abstract

Background: As a major tumor suppressor gene, P53 plays a principal role in the apoptosis of cancer cells. Harmine is a harmal-derived alkaloid with antioxidant and anticancer properties. This study was designed to assess the ability of harmine to express P53 gene and stimulate apoptosis in breast cancer cell line.

Methods: MCF−7 cell line was cultured and treated with harmine. Half maximal inhibitory concentration (IC50) assay was carried out through MMT method following 24 h of treatment. Flow cytometry technique was employed to measure apoptotic cells and real−time PCR was performed to estimate P53 gene expression in tumor cells.

Results: The IC50 for the harmine extract in MCF−7 cells was 30 µM. Harmine administration in all treated groups resulted in a significant increase in apoptosis and P53 gene expression in MCF−7 cells ($P < 0.00001$).

Conclusions: It seems that harmine administration is able to induce apoptosis in MCF−7 cells through the up−regulation of P53 expression.

Keywords: Harmine, P53 gene, MCF-7, Apoptosis, Cell line

Introduction

Harmine is an active component of *Peganum harmala* and an herbal alkaloid of the beta-carboline family.[1] *Peganum harmala* is a member of the Zygophyllaceae family that grows in many countries in North Africa and the Middle East. This component, which contains alkaloids, is generally found in the seeds and root of *Peganum harmala*.[2] Harmine and Harmaline are the most important alkaloids available in *P. harmala* with different effects.[3] Among the clinical applications of the plant essence, angiogenesis process, bradycardia and low blood pressure are the most important examples. Moreover, this plant essence has antiallergic, antispasm and antiadrenergic effects.[4]

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Based on the documentations in traditional medicine, this plant used to be employed as an appetizing substance and antiparasitic agent, and further involved in soporific emmenagogue.[5] Additionally, it is a strong controller of tyrosine phosphorylation-regulated kinase and shows cytotoxic activity against tumor cells in the human body.[6] Harmine can induce apoptosis and regulate transcription factors and pro-inflammatory cytokines.[7] Moreover, it can suppress TNF-α activity as well as nitrite oxide production,[8] and has shown little effect on noncancerous cells.[9] The protective effect of harmine on the periodontal ligament and human neural cells has been reported.[10,11] The results of a study by Geng et al. showed that harmine inhibited cancer cell growth through the coordinated activation of apoptosis and inhibition of autophagy.[12] The molecular mechanism of harmine anticancer effects is not completely fathomed. Breast cancer is the most common cancer among women worldwide.[13] As a human breast carcinoma, MCF-7 cell line has a wild-type P53 gene which cannot induce apoptosis. P53 gene is the most important tumor suppressor gene whose inactivity can be due to epigenetic changes or mutations caused in this gene. More than half of the cancer cases often have mutations in P53 gene.[14] Herbal medicines are the basis of the anticancer potential of various chemotherapeutic agents.[15] Regarding the growing occurrence of breast cancer, numerous studies have been conducted on the diverse cell line of breast cancer. Given the therapeutic properties of harmine and the frequency of breast cancer, the present study investigated the effects of harmine on P53 gene expression and subsequent apoptosis in MCF-7 cell line.

Materials and Methods

The cell line, culture, and study design

In this experimental study, MCF-7 cells (Pasteur Institute national cell bank) were cultured in RPMI-1640 medium (Sigma) complemented by 1% streptomycin-penicillin (Sigma) and 10% FBS (Sigma). Then the MCF-7 cells were incubated at 37°c (moistened atmosphere covering 5% CO_2). Harmine was dissolved in stock solutions, and a proper concentration was created through the harmine dilution in DMSO (2%) according to the reported procedures. When cells became >80% confluent and grew exponentially in 10 cm diameter culture dishes, 10^5 cells (MCF-7) were counted. They were then plated in 3-cm diameter culture dishes for 24 h in RPMI-1640

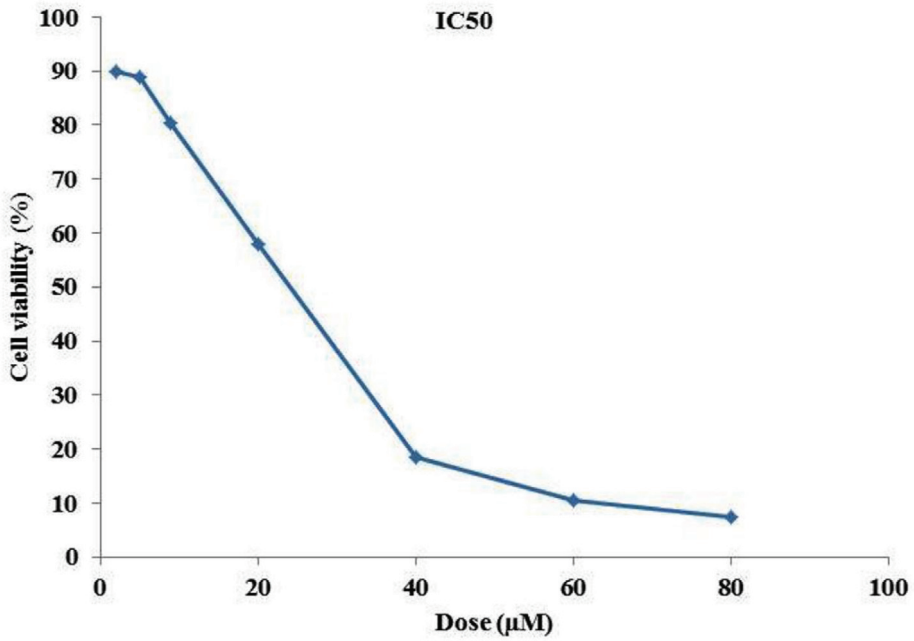

Figure 1. IC50 analysis of harmine in MCF-7 cell lines following 24 h of treatment.

culture medium prior to incubation with certain concentrations of harmine, based on IC50 index, and at different times (24, 48, and 72 h).[13]

Chemicals

To obtain the desired doses, harmine (7-methoxy-1methyl-9h-pyrido) powder (Sigma, USA) with the formula ($C1_3H_{12}N_2O$) was diluted in Dimethyl sulfoxide (DMSO) (Sigma, USA) 2% with the formula $(CH_3)_2SO$.

IC50 analysis

The IC50 standards for the harmine in MCF−7 cells were attained after 24 hours of treatment. Next, 10,000 cells were counted, located in each well of a 12-well microplate, and treated with different harmine concentrations (0, 10, 20, 30, 40, 50, 60, 70, and 80 µM). To calculate the cell viability, MTT survival test was performed as stated by the available protocols. A diagram of viability against extract concentration was used to evaluate IC50 values for MCF−7 cell line.[14]

Flow-cytometric examination

The ratio of apoptotic cells was measured by means of flow cytometry subsequent Annexin V−FITC and propidium iodide staining. A minimum of 5×10^5cells/mL were analyzed for each sample. MCF−7 cells were treated with harmine for 24, 48, and 72 h, washed in PBS, and resuspended in binding buffer (10X; 5 µl). Annexin V−FITC was added to cell suspensions, and the examination was subsequently performed in accordance with the manufacturer's protocol. Ultimately, the apoptotic cells were calculated through FAC Scan flow cytometry (Becton Dickinson, Germany).[16]

Real-time PCR assay

P53 mRNA expression levels in MCF−7 cells were measured following treatment with harmine at different time points. In the initial step, total RNA was isolated by RNeasy mini kit and treated by RNase−Free DNase set (Qiagen) to remove the genomic DNA according to the manufacturer's instructions. Via DNase set kit, the extracted RNA samples were treated to eliminate the genomic DNA. The cDNA version was produced from the extracted RNA related to the previous step by means of RevertAid™ First Strand cDNA Synthesis Kit. The expression level of the given gene was measured through GAPDH primer (Glyceraldehyde 3- phosphate dehydrogenase) as endogenous control by Maxima SYBR

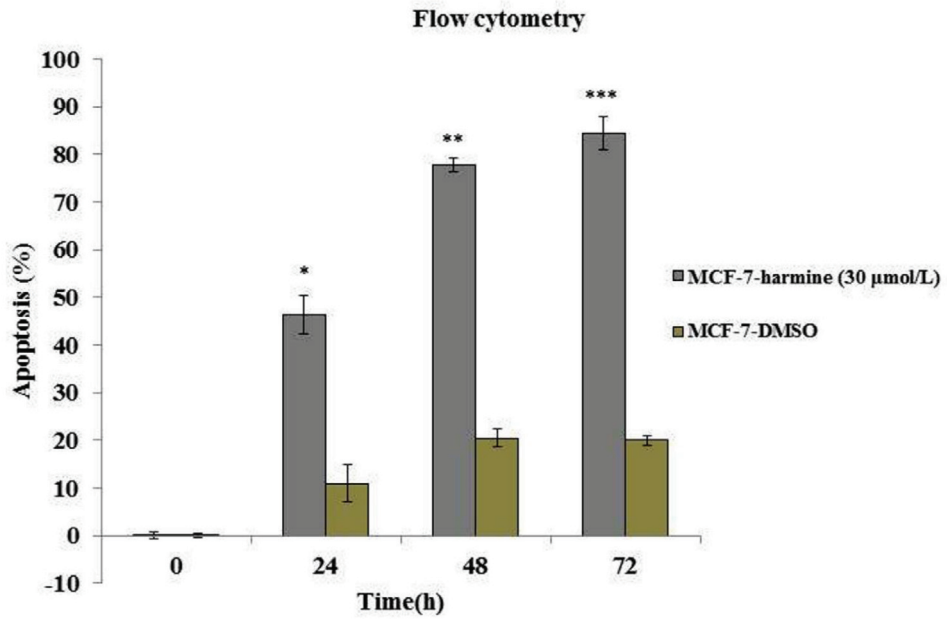

Figure 2. Relative levels of apoptotic cells in MCF-7 cells treated with 30 µM harmine for different times. The percentage of apoptotic cells was measured using AnnexinV FITC and PI assay. *Significant increase in all harmine groups compared to the DMSO control groups (P<0.00001).

Table 1. Primers used in real-time PCR

Primer sequences	Primer ID
AAGCTCATTTCCTGGTATG	GAPDH-F
CTGCCACAAGAACTAGAGGATAAGA	GAPDH-R
CGAGTCCCGCGGTAATTCTT	P53 -F
TGCAGAAGAGGTGCAAGACC	P53-R

GAPDH: Glyceraldehyde3-phosphatedehydrogenase, as endogenous

Green/Rox qPCR master mix (Fermentas co) through Comparative Ct (ΔΔ Ct) technique. First, denaturation at 95°C for 10 min, denaturation at 95°C for 15 sec, and annealing temperatures at 60°C for 1 min with 40 cycles were performed, and the melt curves (increment 3.0°C, 60°C → 95°C) were then drawn by Stepone plus (Applied biosystem). These tests were done in triplicate and independently repeated for at least three times. The sequence of the employed primers is shown in table 1.[13]

Statistical analysis

After extracting the information, the Kolmogorov-Smirnov test was first conducted to confirm the data compliance of the normal distribution. One-way analysis of variance (one-way ANOVA) was used for statistical analysis and Tukey post hoc test was applied to determine the difference between the groups. SPSS 16 was used for data analysis, the results were expressed as a mean±standard error, and $P<0.05$ was considered significant.

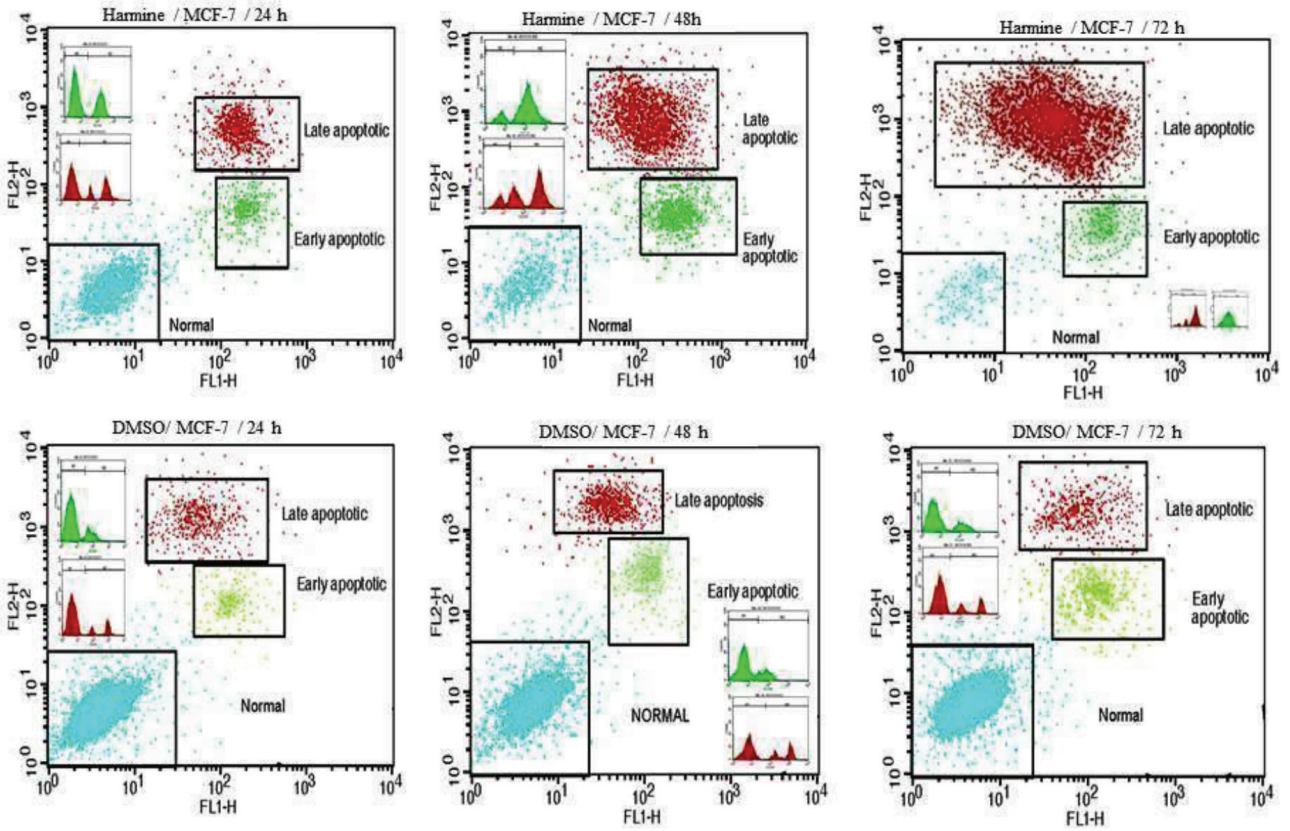

Figure 3. Histogram of the early and late apoptosis of MCF-7 cells treated with harmine for different times. Cells incubated with the vehicle DMSO were used as the control.

Results

IC50

The dark blue formazan crystals were observed in MCF−7 cells due to the treatment of cells with MTT solution, suggesting their metabolic action. The IC50 value for the harmine extract was established. The results showed that 30 µm harmine was required to attain the half−maximal inhibitory concentration in MCF−7 cells at 24 h (Figure 1).

Evaluation of early and late apoptosis

The flow cytometry outcomes displayed that harmine at different time points (24, 48, and 72 h) was able to influence apoptosis in MCF−7 cells. Harmine stopped MCF−7 cells proliferation (46.28% of inhibition) at 24 h, (77.75% of inhibition) 48 h, and (84.4% of inhibition) 72 h (Figure 2). The results of apoptotic cells measurement showed a significant increase in all harmine treated groups compared to the DMSO control groups (P<0.00001) (Figure 3).

Real-time PCR

Following harmine administration, P53 gene expression was up−regulated and significantly increased in all harmine groups compared to the control group (P<0.00001) (Figure 4).

Discussion

In the present study, the effect of harmine on MCF-7 cell line was investigated. The results indicated that harmine treatment induced apoptosis in MCF−7 cells through the up−regulation of P53 expression. For the first time, we reported that harmine is able to up−regulate p53 gene expression in breast cancer cell line. To recognize the inhibitory effect of harmine in MCF-7 cells, diverse molecular methods were employed. Furthermore, harmine induced apoptosis in breast cancer cells in a time−dependent manner, and real−time PCR outcomes on breast cancer cells showed that apoptotic induction in MCF−7 cells by harmine requires the activation of P53 gene expression. It was further observed that P53 gene expression in all examined time points (24, 48, and 72 h) increased significantly compared to the DMSO control group.

Primarily, we studied the expression of P53 in harmine-mediated apoptosis using real-time

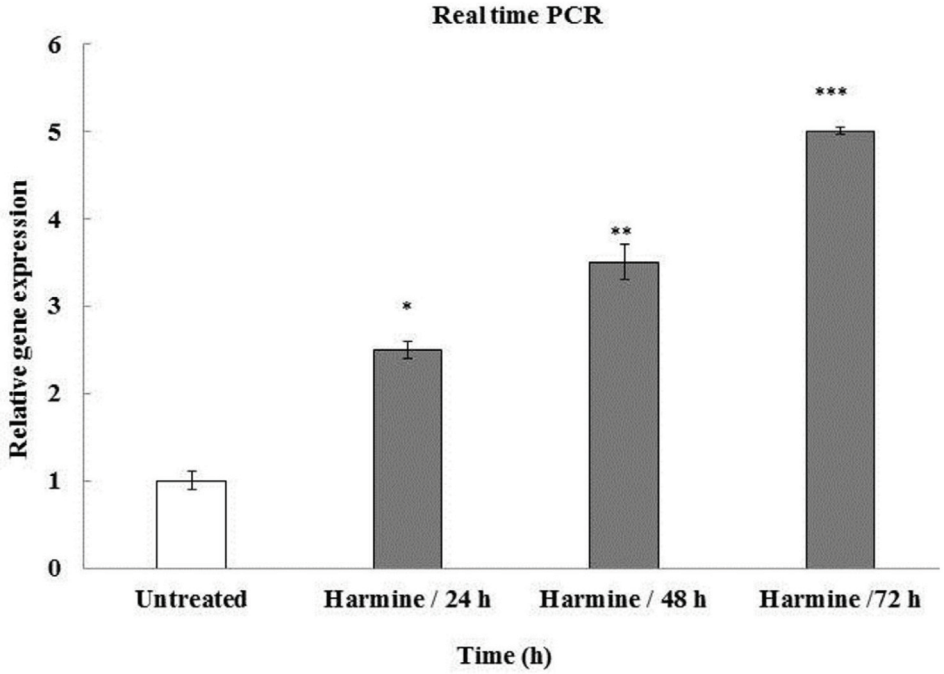

Figure 4. P53 transcript levels in MCF-7 cells after treatment with harmine compared to untreated cells. *Significant increase in all harmine groups compared to the control group (P<0.00001).

PCR, which results indicated that harmine was responsible for the over-expression of P53 in a time-dependent manner in MCF-7 cells. Harmine molecular mechanisms are not completely clear. While more than half of human tumors often have mutations in P53 alterations in tumor suppressor genes, they are not always due to mutations and may also be due to such epigenetic alterations.[16] Hyperacetylation of P53 can also cause the hyperactivity of this protein. Inactivation of P300 is encountered in several types of tumors such as MCF-7 cell line.[13] The human breast carcinoma cell line MCF-7 has a wild-type P53, which is responsible for the epigenetic event (hypoacetylation P53), yet is not functional and cannot induce apoptosis.[14] It has been evidenced that the pharmacologic activation of P300 might promote apoptosis by the direct hyperacetylation of P53 in cancer cells and could have anticancer effects.[13] Our results are consistent with the results of Zhao et al. who showed that harmine inhibited the telomerase activity of MCF-7 cells by down-regulating hTERT mRNA expression accompanied by an accelerated senescent phenotype.[17]

In this study, treatment by 30 µmol/L of harmine at various times showed a time-dependent increase in apoptotic cell count of the cancerous cells as measured by flow-cytometric assay. It seems that harmine induces apoptosis in p53-dependent or independent pathways.[18] Dai et al. observed that harmine inhibited angiogenesis and suppressed tumor growth through the activation of p53 in endothelial cells.[19] Harmine may induce its anticancer effects by triggering G1 arrest, apoptosis, and p53-independent up-regulation of p21.[20] Apoptosis is associated with cancer as a physiological pathway for regulating cellular morphogenesis and homeostasis.[13] Harmine seems to induce cell cycle arrest and has antiproliferative and pro-apoptotic effects in cancer cells.[21] Due to the expression of the p53 gene, harmine induces apoptosis in human lung cells.[22] The influences and efficient mechanisms of the harmine were studied at the concentrations defined by IC50 on the breast cancer cell line. In the present study, it was assumed that harmine might promote apoptosis through directly acetylating P53 in certain cancer cells. Moreover, harmine has various effects on apoptosis induction in normal and cancer cells, such that it could be used as an anticancer strategy. It seems that harmine is able to acetylate and activate P53 and induce apoptosis in response to DNA damage in some cancer cells. However, the outcomes of some studies have shown that the antitumor properties of harmine might occur via one or more of mechanisms such as cytotoxicity and antioxidant activity. Our results are in line with the findings of Li et al. who indicated that harmine has pro-apoptotic and antineoplastic properties.[25] Cao et al. concluded that harmine has strong antiproliferative effects, which is also in accordance with the current research.[24] In one study, it was observed that harmine activated caspases 8, 9, and 7 in a dose-dependent manner in breast cell cancer.[27] Furthermore, Hamsa et al. reported that harmine activated the intrinsic and extrinsic pathways of apoptosis in B16F-10 melanoma.[26]

In general, the results of this study showed that harmine administration inhibited the proliferation of MCF-7 cells in a time-dependent manner. Moreover, for the first time, we observed that harmine probably induced apoptosis through the P53 gene stimulation pathway in the breast cell line. In this regard, further studies are required to evaluate the effect of harmine on the inhibition and treatment of breast cancer.

Conclusion

The results displayed that harmine effects were significant at all treatment times. Eventually, harmine treatment for 72 hours induces the highest apoptosis through P53 gene expression and its apoptotic activities. Due to the proven remedial properties of harmine, it is possible to establish a new therapeutic approach based on this plant against the cancer cells.

Acknowledgements

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Conflict of Interest

None declared.

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