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Antiproliferative and apoptosis-inducing effects of *Peganum harmala* L. seed extracts on gastric adenocarcinoma

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ABSTRACT

Introduction: Gastric cancer treatment remains challenging due to side effects and limited options. This study investigated *Peganum harmala* L., a traditional medicinal plant with anticancer alkaloids, by evaluating its crude extract and fractions for antiproliferative, apoptotic, and cell cycle effects on human gastric adenocarcinoma (AGS) cells.

Methods: Fractionation of the crude hydroalcoholic extract was done using solvents of varying polarity (chloroform, ethyl acetate, n-butanol, and n-hexane). Antiproliferative effects were assessed via MTT assay at five concentrations (0-200 µg/mL) on AGS and human dermal fibroblast (HDF) cells. Apoptosis and cell cycle were conducted via flow cytometric technique after 48 h treatment by annexin V-FITC/propidium iodide binding and propidium iodide intercalation into nuclear DNA, respectively.

Results: The crude extract and three fractions (chloroform, n-butanol, ethyl acetate) showed selective cytotoxicity against AGS cells versus HDFs ($P<0.05$). The n-butanol fraction indicated the highest potency compared to the crude extract and other fractions ($P<0.05$). Apoptosis analysis via flow cytometry indicated a noticeable increase in apoptosis in AGS cells treated with *P. harmala* crude extract, compared to untreated cells ($P<0.05$). Flow cytometric analysis revealed that both the crude extract and n-butanol fraction significantly arrested cells in the G2/M phase while reducing the G1 phase population ($P<0.05$).

Conclusion: *P. harmala* seed extracts demonstrate significant, selective antiproliferative effects on gastric cancer cells, mediated partly through the induction of apoptosis and arresting the cell cycle in G2/M phase. These findings validate traditional uses and highlight the n-butanol fraction as particularly promising for further anticancer development.

Implication for health policy/practice/research/medical education:
Peganum harmala seed extract selectively targets gastric cancer cells with minimal harm to normal cells. Among the crude extract and other fractions, the n-butanol fraction showed the strongest activity, inducing apoptosis and cell cycle arrest. This suggests it is a promising natural adjuvant therapy with fewer side effects than conventional treatments.

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Introduction

As a devastating global health issue, gastric cancer continues to be responsible for a substantial annual burden of cancer-related fatalities worldwide (1-3). Despite advances in conventional treatments like surgery, radiotherapy, and chemotherapy, therapeutic outcomes remain unsatisfactory due to limited efficacy and severe side effects (4,5). This urgent unmet need has driven the search for novel anticancer agents, with medicinal plants emerging as a promising source (6-8). The rich

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phytochemical diversity of plant species offers a vast reservoir of structurally unique compounds that may overcome the limitations of current therapies (9). The therapeutic use of medicinal plants is deeply rooted in global traditional medicine systems (10,11). Naturally occurring compounds from plants exhibit diverse mechanisms against cancer cells, offering potential advantages over synthetic drugs (12,13). Among these mechanisms, apoptosis has become a key target for anticancer drug development (14-16). The inherent capacity to trigger programmed cell death with a high degree of selectivity toward malignant cells makes plant-derived compounds particularly attractive (17). Recent studies highlight that plant-mediated apoptosis often involves modulation of proteins related to the Bcl-2 family, activation, and mitochondrial pathway regulation, providing multiple therapeutic targets (18-20).

Many plant-derived compounds have already transitioned into clinical use or show significant potential (21,22). Notable examples include paclitaxel from *Taxus brevifolia* and camptothecin from *Camptotheca acuminata*, which revolutionized cancer chemotherapy (23). *Peganum harmala* L. (Zygophyllaceae), known as "Espand" in Iran and "Harmel" in North Africa, exemplifies these potentials (24,25). Various parts of this plant, particularly its seeds and roots, have been used traditionally across Iran, Turkey, and China to treat diverse conditions, including rheumatism, respiratory disorders, and metabolic diseases (26,27). The plant's pharmacological activity stems primarily from its β-carboline and quinazoline alkaloids, which demonstrate broad biological effects, including notable anticancer properties (27-29). Recent pharmacological investigations have revealed that these alkaloids exert anticancer effects through DNA intercalation, topoisomerase inhibition, and cell cycle arrest (30,31).

Given the poor prognosis of gastric cancer and the limitations of current therapies, we focused on evaluating *P. harmala* seed extracts. The present research was designed to evaluate the antiproliferative and apoptosis-inducing activities of the crude hydroalcoholic extract, along with four subsequent fractions, in AGS gastric cancer cells using *in vitro* assays.

Materials and Methods

Plant material and authentication

Commercially available *P. harmala* L. seeds were obtained from a local market in Iran. The plant species was taxonomically authenticated as *P. harmala* L., and a voucher specimen (MPSKUMS-188) has been archived in the herbarium of the Medical Plants Research Center at Shahrekord University of Medical Sciences (SKUMS).

Extraction and fractionation

The seeds were ground to a fine powder and subjected to triple maceration in 80% ethanol for 96 hours at room temperature. The pooled extracts were filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator with the water bath set at 40 °C.

For fractionation, the crude extract was suspended in ethanol-water (1:1) and sequentially partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol (Merck, Germany) by liquid-liquid extraction. The aqueous residue was concentrated separately. All fractions were stored in sterile containers at 4 °C.

Working solutions (25 mg/mL) were prepared in DMSO (Samchun, Korea) and diluted in culture medium, maintaining final DMSO concentrations below 0.2% (v/v) - a level confirmed not to affect cellular viability (32).

Cell culture and viability assay

The AGS gastric adenocarcinoma cell line (Pasteur Institute, Iran) and human dermal fibroblasts (HDFs, from SKUMS) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 0.25 µg/mL amphotericin B (Gibco), 100 µg/mL streptomycin (Sigma, USA), and 100 U/mL penicillin at 37 °C in 5% CO_2. For viability assessment, cells (6×10^3/well) were seeded in 96-well plates (SPL, Korea) and treated with extract/fractions (0-200 µg/mL) for 72 hours. MTT assays were performed by adding 60 µL of MTT (1 mg/mL in PBS; Sigma) per well, incubating for 4 hours, and solubilizing formazan crystals with acidic isopropanol (Merck). Absorbance was determined at 570 nm by a microplate reader (STAT FAX 2100, USA). Viability of cells was computed as: Cell viability= (Absorbance of treated cells/ Absorbance of control) × 100.

IC_{50} values were determined through probit regression analysis (SPSS v16).

Analysis of apoptosis by the flow-cytometry technique

AGS cells (1×10^5/well) were treated with the crude extract (46 µg/mL, equivalent to IC_{50}) for 48 hours in 6-well plates. Cells were collected and subjected to a dual-staining protocol utilizing Annexin V-FITC and propidium iodide for apoptosis detection (BD Biosciences, USA) in binding buffer for 20 minutes (dark, RT), and analyzed using a flow cytometer (Partec, Germany) following manufacturer protocols. Early and late apoptotic populations were quantified using FlowJo software.

Analysis of the cell cycle via the flow-cytometry technique

Cell cycle analysis was conducted by the BD Cycletest™ Plus DNA Kit (BD Pharmingen, USA) according to the manufacturer's instructions. Briefly, cells were permeabilized with the supplied trypsin buffer, followed by fixation and RNA digestion using a methanol-based solution containing RNase. The cells were then stained with propidium iodide (PI) for 3 hours in the dark. DNA content was measured using a flow cytometer

(BD FACSCalibur) with a linear scale, and the resulting histograms were analyzed to ascertain the percentage of the cell population in each phase of the cell cycle [G0/G1 (G1), S, and G2/M (G2)].

Results

Antiproliferative effects of *Peganum harmala* seed extract

The MTT assay results demonstrated concentration-dependent growth inhibition of AGS gastric cancer cells following treatment with the crude hydroalcoholic extract (0-200 µg/mL for 48 hours). Notably, the extract exhibited selective cytotoxicity, showing significantly greater potency against AGS cells (IC_{50} = 45.8 ± 5.3 µg/mL) compared to normal HDFs (IC_{50} = 197.1 ± 4.15 µg/mL, $P < 0.05$) (Figure 1, Table 1). This selectivity suggests potential therapeutic safety margins.

Fraction-specific cytotoxic activity

All active fractions maintained selectivity for cancer cells, with IC_{50} values against HDFs ranging from 176.1 to 407.4 µg/mL (Table 1). Fractionation revealed distinct biological activities:

- The n-butanol fraction displayed the strongest anticancer effect (AGS IC_{50} = 20.1 ± 6 µg/mL)
- Ethyl acetate (IC_{50} = 35.4 ± 6.4 µg/mL) and chloroform (IC_{50} = 38.9 ± 8 µg/mL) fractions showed intermediate activity
- The n-hexane fraction exhibited no significant cytotoxicity ($P > 0.05$; Figure 2)

Analysis of apoptosis induction by crude extract

Flow cytometric evaluation of AGS cells following a 48-hour treatment with the crude extract of *Peganum harmala* (46 µg/mL) demonstrated a substantial pro-apoptotic effect. The scatter plots (Figure 3) delineate distinct cell populations. Quantitative analysis of these populations indicated that a combined total of 22.28% of the treated cells were undergoing apoptosis, distributed between early and late apoptotic stages. The population of necrotic cells (Annexin V-/PI+) remained minimal, accounting for less than 5% of the total, which was comparable to the untreated control. This significant shift towards apoptosis in treated cells compared to the control group was statistically confirmed ($P < 0.05$), underscoring the efficacy of the crude extract in triggering programmed cell death.

Table 1. Cytotoxic potential of *Peganum harmala* seed extracts against gastric cancer (AGS) and normal fibroblast (HDF) cell lines

Extract/fraction	Cell lines	
	AGS (µg/mL)	HDFs (µg/mL)
Crude extract	45.8±5.3	197.1±4.15
n-Hexane fraction	>1000	>1000
Chloroform fraction	38.9±8	242.4±12.6
n-Butanol fraction	20.1±6	407.4±10.6
Ethyl acetate fraction	35.4±6.4	176.1±8.9

Values represent IC_{50} (µg/mL) determined by MTT assay after 48-hour treatment. Data were analyzed using probit regression (SPSS v16). Lower values indicate greater potency.

Effect of various fractions of *Peganum harmala* on apoptosis in AGS and HDF cells

As illustrated in Figure 4, treatment with *P. harmala* fractions induced apoptosis in both AGS and HDF cell lines. However, the extent of apoptosis was significantly higher in AGS gastric cancer cells compared to normal HDF cells ($P < 0.05$). Among the tested fractions, the n-butanol fraction exhibited the most potent pro-apoptotic effect on

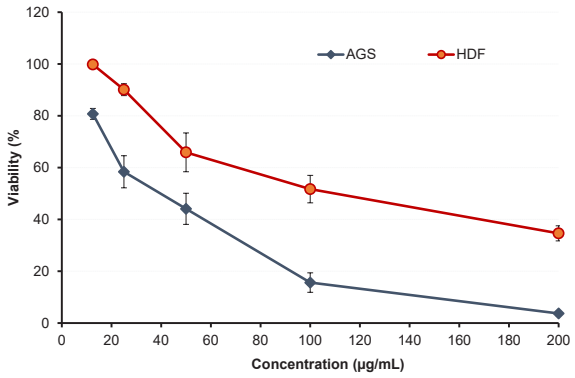

Figure 1. Dose-response curves of *Peganum harmala* crude extract in AGS (cancer) and HDF (normal) cell lines. Cells were exposed to increasing concentrations (0-200 µg/mL) for 48 hours. Points show mean viability ± SEM (n = 3). The extract demonstrated significantly greater cytotoxicity in cancer cells ($P < 0.001$, probit regression).

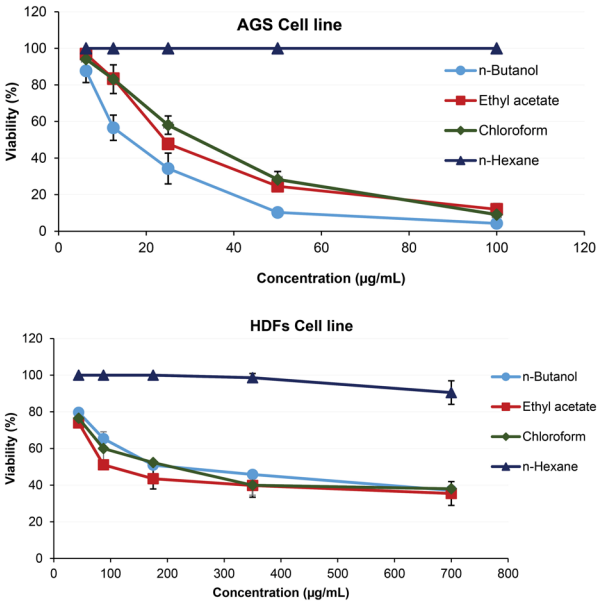

Figure 2. Comparative cytotoxicity of *Peganum harmala* fractions. The n-butanol (■), ethyl acetate (●), and chloroform (▲) fractions showed dose-dependent growth inhibition in AGS cells, while n-hexane (×) exhibited minimal activity. Data represent mean ± SEM from triplicate experiments.

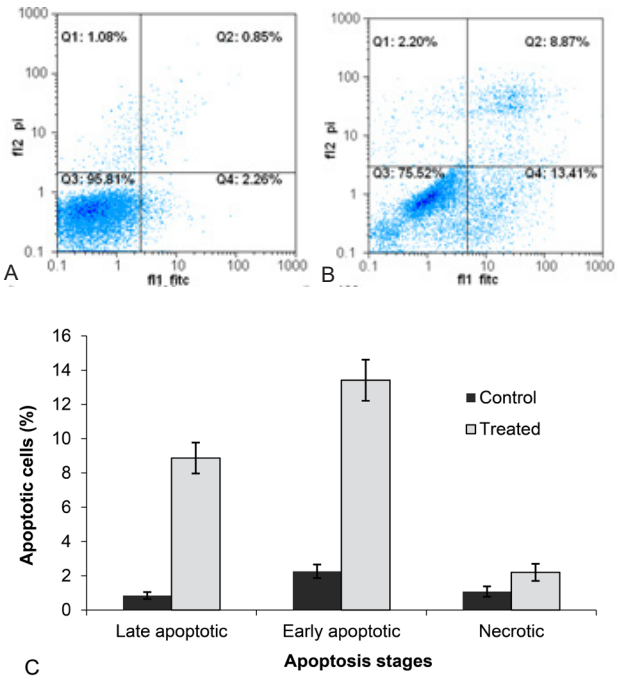

Figure 3. Apoptosis induction by *Peganum harmala* crude extract in AGS cells. (A) Untreated control showing viable cells; (B) Treated cells (46 µg/mL, 48 h) demonstrating apoptotic populations; (C) Quantification revealing 22.28% apoptosis. A noticeable induction of apoptosis was observed in AGS cells treated with *P. harmala* crude extract, as compared to the untreated cells ($P < 0.05$).

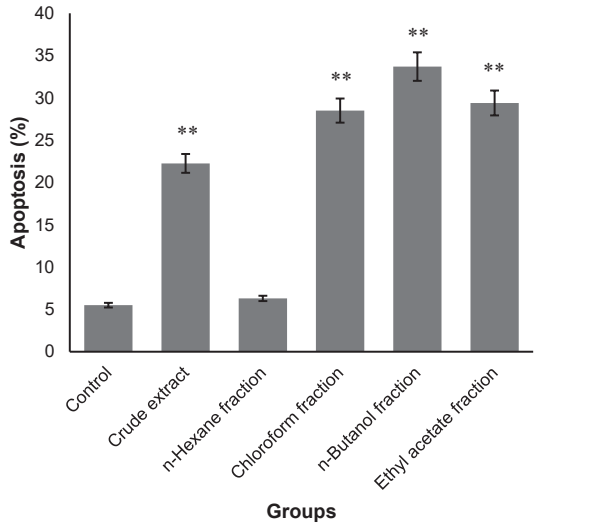

Figure 4. Apoptosis induction in AGS cells treated with Peganum harmala crude extract and fractions. Cells were treated with the specified concentrations of the crude extract, as well as chloroform, n-butanol, n-hexane, and ethyl acetate fractions for 24 hours. Quantitative assessment of apoptosis, conducted via staining by Annexin V/PI (evaluated by flow cytometry technique), revealed that the treatment induced significant cell death. All values represent the mean ± SD (n=3). Statistically significant differences from the control are indicated as follows: $^{**}P < 0.01$.

AGS cells, showing a statistically significant increase in apoptosis compared to other fractions ($P<0.05$; Figure 4).

Effect of *Peganum harmala* fractions on the cell cycle in AGS cells

Cell cycle analysis, presented in Figure 5, revealed that only the crude extract and the n-butanol fraction of *P. harmala* caused significant alterations in the cell cycle distribution of AGS cells. Specifically, these treatments led to a decrease in the proportion of cells in the G1 phase and a concomitant increase in the G2/M phase population compared to the control group ($P<0.05$). Conversely, the other fractions tested did not induce any statistically significant changes in the cell cycle phases ($P>0.05$; Figure 5).

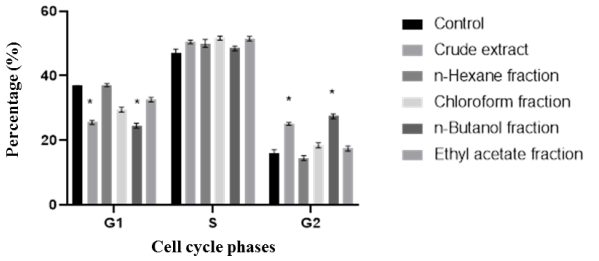

Figure 5. Cell cycle distribution in AGS cells following treatment with Peganum harmala crude extract and its fractions. AGS cells were exposed to the crude extract and various fractions for 24 hours. The DNA content was determined via the flow-cytometric technique. The cellular distribution across the various cell cycle phases—G1, S, and G2/M—is presented herein. Data represent the mean ± SD of triplicate experiments. Statistically significant differences from the control are indicated as follows: $^{*}P < 0.05$.

Discussion

In this investigation, we evaluated the antiproliferative effects of a hydroalcoholic crude extract and four corresponding fractions from *P. harmala* L. seeds on the AGS gastric adenocarcinoma cell line. In vitro cytotoxicity assays demonstrated that the crude extract significantly reduced cell viability in a dose-dependent manner. Probit regression analysis revealed statistically significant differences in the antiproliferative activity of the crude extract across the tested concentrations. Among the fractions of *P. harmala* L., the n-butanol fraction exhibited superior growth inhibition against the AGS cell line, demonstrating a lower IC_{50} value than the crude extract and the other fractions. These observations align with previous studies in which fractionation yielded fractions with enhanced cytotoxic potential against malignant cell lines (33,34). Collectively, these results suggest that fractionation of plant-derived crude extracts can enhance their antiproliferative activity while reducing the required effective concentration.

Our findings demonstrated that *P. harmala* L. seeds' crude extract induced cell death primarily through early apoptosis. This is consistent with the study by Bournine

et al, who reported that *P. harmala* extract significantly increased the percentage of apoptotic cells in gastric cancer cells, particularly after 48 hours of incubation (35). This observation corroborates the work of Hashemi et al., who reported that *P. harmala* L. seeds extract triggered apoptotic cell death in breast cancer cells via both intrinsic and extrinsic pathways (36). These collective findings provide compelling evidence for the cytotoxic potential of *P. harmala* and its capacity to induce programmed cell death in malignant cells while suppressing their proliferation.

The observed G2/M phase arrest induced by both the crude extract and the n-butanol fraction provides a crucial mechanistic insight into the antiproliferative activity of *P. harmala*. This arrest often serves as a critical checkpoint, allowing the cell to initiate repair mechanisms; however, if the damage is irreparable, it can directly trigger apoptotic pathways (37). The concomitant induction of G2/M arrest and apoptosis, as seen in our study, suggests that the bioactive compounds in *P. harmala* may cause DNA damage or mitotic spindle defects, ultimately pushing the cancer cells toward programmed cell death (38). This correlation between cell cycle arrest and apoptosis has been previously reported for other plant-derived anticancer agents. For instance, β-carboline alkaloids, which are key constituents of *P. harmala*, have been shown to induce G2/M arrest and subsequent apoptosis in various human cancer cells (39). AlQathama and Prieto demonstrated that harmine, a key β-carboline alkaloid in *P. harmala*, induced morphological changes and DNA fragmentation consistent with apoptosis in melanoma cells (40). Another potential mechanism has been suggested by Fan et al., indicating that compounds in *P. harmala*, particularly in polar fractions, may inhibit the PI3K/Akt pathway, which is frequently activated in cancer cells to suppress apoptosis and promote proliferation (41). Moreover, these extracts exhibited cytotoxicity in animal models such as BALB/c mice (42). Isolated β-carboline alkaloids from *P. harmala* have also demonstrated inhibitory effects against Lewis lung carcinoma, sarcoma-180, and HepA tumors in murine models (43). Mechanistic studies indicate that certain β-carbolines can intercalate DNA and inhibit topoisomerase activity, thereby inducing cytotoxicity (44). Previous investigations into *P. harmala* have also highlighted the bioactivity of its phenolic-rich fractions, particularly their capacity to inhibit cell migration and angiogenesis. In one such study, Kemel and colleagues demonstrated that an n-butanol (n-BuOH) extract derived from the seeds of *P. harmala* effectively suppressed cellular proliferation, migration, and angiogenic processes. This same extract was characterized by potent antioxidant and free radical-scavenging activities. Furthermore, it induced significant cytotoxic effects in MCF-7 cells of breast cancer (45). Therefore, the G2/M arrest observed in AGS cells is not an isolated event but is likely intrinsically linked to the apoptosis induction we reported, collectively contributing to the potent cytotoxic effects of the extract and its active fraction.

Conclusion

The current study indicates that the crude hydroalcoholic extract of *P. harmala* L. seeds effectively suppresses the proliferation of AGS gastric adenocarcinoma cells by inducing early apoptosis. These findings suggest that certain *P. harmala* L. seeds-derived extracts and fractions hold potential as promising candidates for the development of novel antitumor agents. However, further research is essential to identify the precise bioactive compound(s) responsible for this cytotoxic activity and to elucidate their underlying molecular mechanisms of action.

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Declaration of AI-assisted tools in the writing procedure

The authors used the DeepSeek AI assistant (DeepSeek Company) for initial language polishing and grammar checks of the manuscript draft. The AI was used solely for editorial purposes to improve readability and ensure grammatical accuracy. All experiments, data collection and data analysis, results interpretation, and scientific writing were conducted entirely by the authors. The final content and scientific integrity of the manuscript remain the sole responsibility of the authors.

Conflict of interests

There is no conflict of interest

Ethical considerations

The protocol of this study was approved by the Ethical Committee of Shahrekord University of Medical Sciences (ethics code: IR.SKUMS.REC.1402.051).

Authors' contribution

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Data curation: Niloufar Mousiany, Nafiseh Bagherian Khouzani, Majid Asadi-Samani, Mohammad-Taghi Moradi.

Formal analysis: Niloufar Mousiany, Ali Rafieian, Mohammad Reza Khosravi Farsani.

Funding acquisition: Majid Asadi-Samani.

Investigation: Niloufar Mousiany.

Methodology: Niloufar Mousiany, Majid Asadi-Samani, Mohammad-Taghi Moradi.

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Writing–original draft: All authors.
Writing–review & editing: All authors.

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