

## Synergistic anticancer effect of *A. absinthium* extract and Taxol on human liver cancer cell line

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### **ABSTRACT:**

Liver cancer is the third most common cause of cancer death globally. among of the most widely used medicinal plants of the *Artemisia* genus belongs to the Compositae (Asteraceae) family; *Artemisia absinthium* is one of the important species of this genus. In the present study, the ability of *A. absinthium* extract alone and in combination with Taxol to induce apoptosis in HepG2 cell line was investigated. In this experimental study, first *A. absinthium* methanolic extract was prepared, then, the cytotoxic effect of combined use of *A. absinthium* extract with Taxol on HepG2 cells proliferation was investigated using MTT test; DAPI staining and flow cytometry analysis were also used to evaluate apoptosis in treated cells; Changes in the expression level of apoptotic genes were evaluated by Real-time PCR; Quantitative data were analyzed by one-way ANOVA at a significant level of  $p < 0.05$ . The results of the MTT test showed that the combination of *A. absinthium* extract with Taxol inhibits the proliferation of HepG2 cells in a concentration-dependent manner. Morphological observations obtained from DAPI method and the results of flow cytometry analysis showed an increase in the percentage of apoptotic cells in the treatment groups. Analysis of the Real-time PCR showed an increase in the expression of apoptotic genes in the treatment groups. The findings of this study showed that the combined use of *A. absinthium* extract with taxol inhibits the proliferation of HepG2 cancer cells and induces apoptosis.

**Keywords:** Liver cancer, *Artemisia absinthium*, Taxol, Apoptosis

### **INTRODUCTION:**

Cancer disease has affected many people for years; The term "*cancer*" can be described as a set of complex processes, including disrupted cell death and unlimited cell proliferation, which often leads to the formation of malignant tumors and invasion to distant tissues (metastasis) (1, 2). According to the latest statistical surveys, cancer is the second leading cause of death after cardiovascular diseases worldwide and the third leading cause of death in Iran. About 18.1 million new cases of cancer and 9.6 million deaths from cancer have been reported in 2018 (2, 3); It is estimated that the number of reported cases of cancer will increase by about 70% by 2035(3). Liver cancer is one of the most common cancers in the world, so that this cancer is the fifth most common cancer in men and the ninth most common cancer among women. The incidence of liver cancer has increased in the last 25 years and it is expected to double in the next 10 to 20 years, so prevention and treatment of liver cancer is

now a major concern (4, 5). The available treatments for patients with liver cancer are very limited, unfortunately this type of cancer is diagnosed in advanced stages (6). Hepatocellular carcinoma is the most common type of liver cancer and the third leading cause of mortality due to cancer. This type of cancer is usually non-removable and non-surgical due to its rapid growth and metastasis, but if diagnosed early, surgical method may be possible for people with the disease. Unfortunately, only between 10 and 30% of people with this type of cancer are eligible for surgery (6, 7). Unfortunately, the methods of chemotherapy and surgery are not effective for patients with advanced hepatocellular carcinoma due to tumor recurrence, metastasis, and poor response to chemotherapy and radiation therapy; therefore, due to the limited treatment methods and resistance of this type of cancer to chemotherapy drugs, the need to create new methods for better treatment of this cancer seems necessary (6, 8). Taxol is one of the drugs used in chemotherapy for liver cancer. Taxol (commonly

called *paclitaxel*) is a compound of di-terpene with a complex structure, which has significant antitumor activity. This drug is an anti-cancer compound with plant origin that is obtained from the bark of the yew tree with the scientific name "*Taxus brevifolia*. L" (9). Taxol prevents microtubule de-polymerization, thus causing tubulin accumulations to become a stable structure that leads to stabilize microtubules and their inefficiency, which ultimately this matter stops the cell cycle at the end of the G2 phase/ M and induces apoptosis (9,10).

The process of apoptosis or programmed cellular death as a conserved method is controlled by genes that is used to remove unwanted or unnecessary cells in living organisms; This process is very important in regulating the growth and proliferation of cells and the incidence of many diseases, such as various kinds of cancers is the result of poor performance or inhibition of programmed cellular death phenomenon (11, 12). Escape from apoptosis is one of the prominent signs of various cancers. Cancer cells actually contain changes that lead to disruption in the apoptotic signal and eventually lead to tumor spread and metastasis (13), as a result, many treatment strategies today are based on restarting planned cell death or apoptosis in cancer cells (12).

Since old time, plants have been an important source for treating a wide range of diseases, because they are complex chemical compounds that are constantly producing natural products and can be an effective source to treat and prevent disease. It has also been found that plants have a wide range of medicinal and biological activities (14-16). It is believed that the anti-cancer effects of plants are caused by suppressing cancer-stimulating enzymes, repairing DNA, stimulating the production of anti-tumor enzymes in the cell, increasing immunity and inducing antioxidant effects (17).

One of the most widely used plants as a medicinal plant of the *Artemisia* genus belongs to *Compositae* (*Asteraceae*) family; which consists of about 500 species worldwide. There are 34 known species of *Artemisia* in Iran. *Artemisia absinthium* is one of the important species of this genus (18, 19). The *Artemisia* genus includes important medicinal plants that are considered worldwide due to their special chemical and photochemical compounds and due to their unique components and compounds such as artemisinin, aromatic compounds, terpenoids, flavonoids, coumarins and phenol derivatives; which helps in its biological activities, especially its antioxidant activity and its effects against cancer. The pharmaceutical industry and cosmetics industry is used in traditional medicine. Researches have shown that extracts of some *Artemisia* species have cytotoxic activity and apoptotic effect in vitro against cancer cell lines and animal cancer models, and in contrast they have low or even ineffective toxicity against normal cells (18,20).

In this way; *Shafi et.al* ( 2012) investigate the role of *A. absinthium* extract in inhibiting cell proliferation and induction of apoptosis in breast cancerous cells

and MDA-MB-231 and MCF-7 lineages . The results of these studies showed the anti-proliferative effects of *A. absinthium* extract on cancerous cells and the activation of apoptotic pathways in both cell lines (21). *Al-Menhali et.al* (2005) investigated the effect of *Thymus vulgaris* plant extract on the proliferation and migration of colon cancerous cells. Their results showed that the extract of this plant can inhibit the proliferation of cancerous cells depending on the concentration and cause to increase apoptosis along with increasing the activity of caspases and also prevents cell migration (22). *Lian et.al* (20018) examined the anti-cancer activity of *Artemisia vulgaris* (*mugwort*) extract against HCT-15 (human colon cancer) cell line and showed that the extract of this plant had a concentration-dependent toxic effect on cancerous cells and causes to inhibit the growth of these cells (23). *Paramee et al* (2018) tested the effects of *Kaempferia parviflora* plant on cell proliferation, migration and cell death in SKOV3 (human ovarian cancer) cells, and the results showed that this plant strongly suppresses cell proliferation and cell migration (24).

The purpose of this experimental study is the combined use of *A. absinthium* extract and Taxol on induction of apoptosis in HepG2 cell line and also the study of changes in the expression level of s "*BAX, Caspase 3,9, p53*" apoptotic gene.

## **MATERIALS AND METHODS:**

### **Materials:**

RPMI culture medium "*BIO-IDEA; Iran*", FBS "*Gibco; USA*", streptomycin-penicillin antibiotic "*BIO-IDEA; Iran*", trypsin "*BIO-IDEA; Iran*", trypan blue solution "*BIO-IDEA; Iran*", MTT powder "*Sigma-Aldrich; USA*", DAPI dye "*Sigma-Aldrich; USA*", Annexin V-FITC kit "*Abcam; UK*", RNA extraction kit "*Pars tous; Iran*", cDNA synthesis kit "*Pars tous; Iran*", Sybrgreen "*Pars tous; Iran*"

### **PROCEDURE:**

#### **Preparation and culture of HepG2 cells:**

HepG2 cell line (human liver cancerous cells) was prepared from *Pasteur Institute of Iran* (NCBICode: C158) and was cultured in RPMI culture medium containing 10% FBS and 1% antibiotics.

#### **Preparation of methanolic extract of *Artemisia absinthium* plant:**

*Artemisia absinthium* plant with identification code 45952 (FUMH) was prepared from the herbarium of Ferdowsi University of Mashhad. To prepare the methanolic extract of *A. absinthium* plant, first the aerial parts of the plant were dried and then powdered, then for each gram of plant powder; 10 ml of methanol was added and placed in the dark at room temperature for 72 hours. During this time the mixture was stirred well 3 times a day. After 72 hours, the resulting mixture was filtered using *What-man* paper and finally extracted by a "rotary evaporator system". To prepare

the main stock for cells treatment, 0.001 g of *A. absinthium* extract was weighed and dissolved in 50 µl of dimethyl sulfoxide (DMSO), then diluted to 1 ml with culture medium. The formula  $N1V1 = N2V2$  was used to construct the treatment concentrations from the main stock (21).

**MTT test to determine cytotoxicity:**

MTT test was used to evaluate the effect of *A. absinthium* extract; Taxol and the combined use of *A. absinthium* extract and Taxol on HepG2 cell proliferation. To do this, first  $4 \times 10^3$  cells were cultured in one 96-well plate after counting by Trypan Blue staining. After the appropriate time has elapsed from cell culture; HepG2 cells were treated at concentrations of 100, 200, 300, 400, 500 and 600 µg/ml of *A. absinthium* extract; concentrations of 2.50, 5, 10, 12.50, 15 and 20 µg/ml of Taxol and concentrations of 100, 200 and 300 µg/ml of *A. absinthium* extract with concentrations of 2.50, 5 and 10 µg per ml of Taxol. After 24 hours of cell treatment, 10 µl of MTT solution was added to the cell medium, then the cell culture plate was incubated for 3 to 4 hours. Then, the supernatant was removed from the cells and 80 µl dimethyl sulfoxide (DMSO) was added to each well and then the plate was transferred to the "EPOCH" spectrophotometer and the light absorption rate of the samples was read at 570 nm (25). The percentage of living cells was calculated by the following formula:

$$\text{Cell viability percentage} = \frac{\text{Absorbance of treated cells}}{\text{Control cells}} \times 100$$

**DAPI Nuclear Staining:**

DAPI staining was used to stain the nucleus of cells, to evaluate apoptosis, to evaluate the density or fragmentation of chromatin in the nucleus of treated cells and to compare it with the control group. To do this, first a sterile cover slip was placed on the bottom of each 6-well plate well, then HepG2 cells were cultured on the cover slips. After the appropriate time has elapsed from culturing and attaching the cells to the cover slips, complete culture medium was added for control group and treatment media (concentration of 300 µg/ml of *A. absinthium* extract; concentration of 12.50 µg/ml of Taxol and concentration of 100 µg/ml of *A. absinthium* extract with a concentration of

10 µg/ml of Taxol) was added for treatment groups slowly from the corner of each plate well. Twenty-four hours after treatment; the supernatant of all groups was removed and 400 µl of methanol was added to the plate as a cell fixative, and finally, the cells were exposed to DAPI dye and changes in the morphology of the cells were examined by fluorescent microscopy (26).

**Annexin V-FITC test:**

This test was used to determine cell death. To perform this test, the "Annexin V-FITC Apoptosis Staining / Detection Kit (ab14085)" was used, in which HepG2 cells were first cultured in 6 –well plate and after 24 hours of cell culture, cells were treated with a concentration of 300 µg per ml of *A. absinthium* extract, a concentration of 12.50 µg/ml of Taxol and a concentration of 100 µg/ml of *A. absinthium* extract with a concentration of 10 µg/ml Taxol. Twenty-four hours after treatment, the cells were separated from the bottom of the plate, transferred to the Eppendorf and then centrifugation was performed. By removing the supernatant, 500 µl of Binding Buffer 1X was added to each sample according to the kit protocol, and then 5 µl of Annexin V and 5 µl propidium iodide were added to each sample. Finally, the samples were incubated in the dark for 5 minutes and then the samples were analyzed by flow cytometry (27).

**Real-time PCR technique:**

The RNA extraction steps were performed according to the "Pars tous" kit protocol and to ensure the appropriate concentration of the extracted RNA, the RNA concentration level was measured by the "EPOCH" spectrophotometer. In continue, the cDNA was synthesized according to the "Pars tous" kit protocol and finally the changes in the expression rate of "BAX, Caspase 3,9, p53" genes in HepG2 cells; in groups treated with a concentration of 300 µg/ml of *A. absinthium* extract; with concentration of 12.50 µg/ml of Taxol and concentration of 100 µg/ml of *A. absinthium* extract with a concentration of 10 µg/ml of Taxol were checked by "Bio-Rad CFX96 RT-PCRs" and "Bio-Rad CFX Manager" software (28,29) . The sequence of primers used in the Real-time PCR technique is as follows:

**Table 1.** Sequence of primers used in Real-time PCR technique

Gene	Sens	Antisense
GAPDH	TGACTTCAACAGCGACACC	TTGCTGTAGCCAAATTCGTT
BAX	TTTGCTTCAGGGTTTCATCCA	CTCCATGTTACTGTCCAGTTCGT
Caspase 3	AGACAGACAGTGGTGTGATG	GTTTCATCCAGTCGCTTTGTGC
Caspase 9	CCAGAGATTCGCAAACCAGAG	CAATGTGAACTTCTGCCGTGA
p53	TTGCCGTCCCAAGCAATGGA	TCTGGGAAGGGACAGAAGATG

**STATISTICAL ANALYSIS METHODS:**

Quantitative data obtained from this experimental study were analyzed in SPSS and GraphPad Prism

software using one-way ANOVA at a significant level of "p <0.05."

Graphs are drawn using Excel software.

To ensure the results of this experimental study; all experiments were repeated three times.

## RESULTS:

### MTT test Results:

The results of MTT test showed that the cell viability rate decreased in a concentration-dependent manner in the treatment groups, as the percentage of cell viability decreased with increasing concentration of *A. absinthium* extract and Taxol. The results of the MTT test also showed that the combined use of *A. absinthium* extract and Taxol had a greater effect on the process of reducing the cancerous cells proliferation of HepG2 cell line than when we used each alone.

By determining the inhibitory concentration of "IC<sub>50</sub>", it was found that this amount was 300 µg/ml for *A. absinthium* extract, 12.50 µg/ml for Taxol and 100 µg/ml of *A. absinthium* extract with a concentration of 10 µg/ml of Taxol for combined use during 24 hours. After performing the MTT test, the "IC<sub>50</sub>" concentrations obtained from this test were used to perform the rest of the tests.

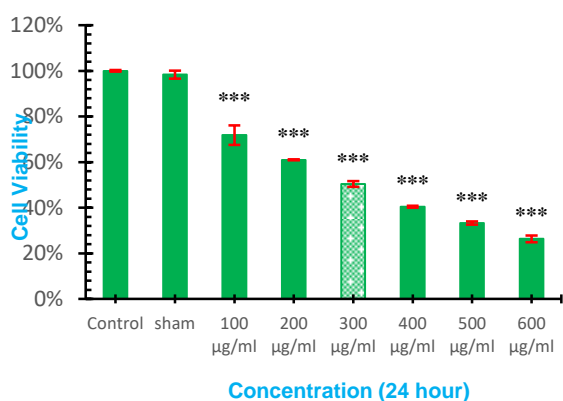


Diagram 1 Comparison of the mean viability percentage of the control sample compared with the samples treated with *A. absinthium* extract in different concentrations in 24 hours ( $p < 0.001$  \*\*\*)

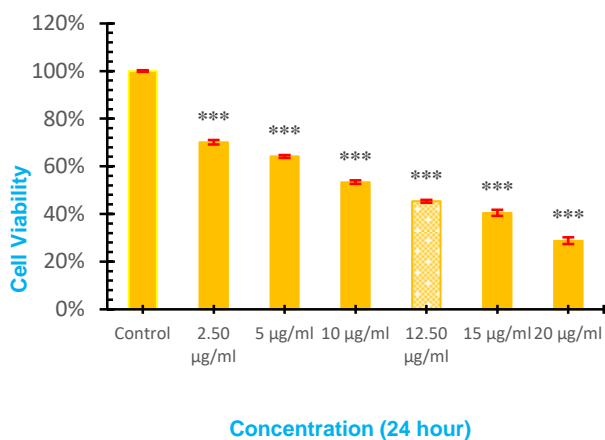


Diagram 2 Comparison of the mean viability percentage of the control sample compared with the samples treated with Taxol at different concentrations in 24 hours ( $p < 0.001$  \*\*\*)

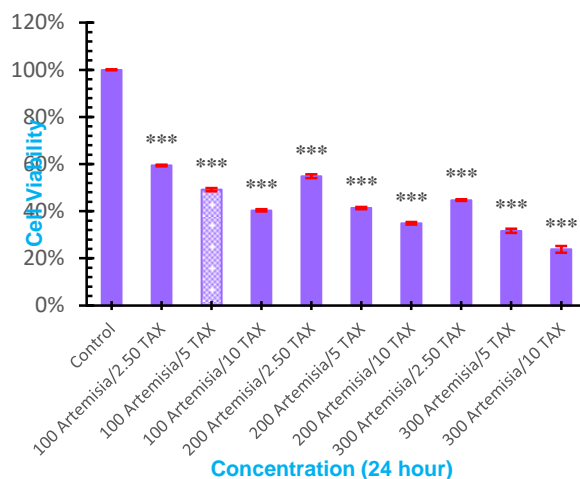


Diagram 3 Comparison of the mean viability percentage of the control sample in comparison with the samples treated with *A. absinthium* extract with Taxol in different concentrations in 24 hours ( $p < 0.001$  \*\*\*)

### DAPI Staining Results:

As shown in Figure 1, the results obtained from DAPI staining show that some morphological changes in the nucleus of HepG2 cells which were placed under treatment with "IC<sub>50</sub>" concentrations of *A. absinthium* extract and Taxol as well as the combined composition of the *A. absinthium* extract and Taxol for 24 hours are seen compared to the nuclei of the control group cells that have not received any treatment. As can be seen in the pictures, the nuclei are fragmented in the treatment groups and the shape of the nuclei is out of normal; which could be a sign of apoptosis incidence in these cells. Examination of morphological changes also showed that some of the treated cells have significant chromatin fragmentation, which these changes indicate morphological changes after 24 hours of treatment. As shown in Figure 1, most cell damage and morphological changes in the nuclei occurred when the cells were treated with a combination of *A. absinthium* extract and Taxol.

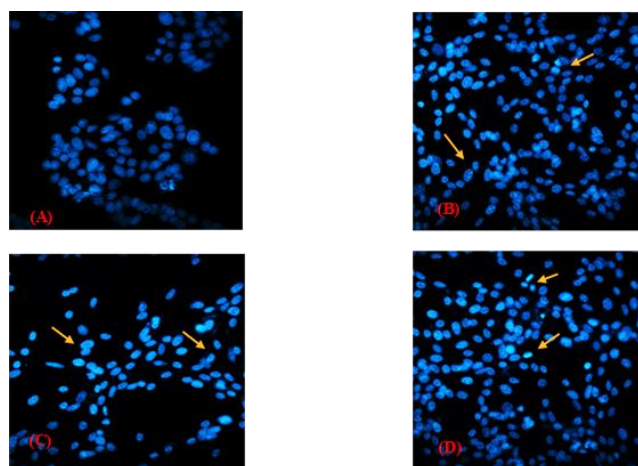


Figure 1. Observation of cell nuclei by DAPI staining after 24 hours

(A) control group; (B) treatment with a concentration of 300  $\mu\text{g/ml}$  of *A. absinthium* extract; (C) treatment with a concentration of 12.50  $\mu\text{g/ml}$  of Taxol; (D) treatment with a concentration of 100  $\mu\text{g/ml}$  *A. absinthium* extract with a concentration of 10  $\mu\text{g/ml}$  Taxol

The tips of the arrows indicate cells that have undergone apoptosis.

(All images are observed at 400X magnification).

### Annexin V-FITC test Results:

To perform this test, HepG2 line cells were treated with "IC<sub>50</sub>" concentrations in 24 hours. As shown in

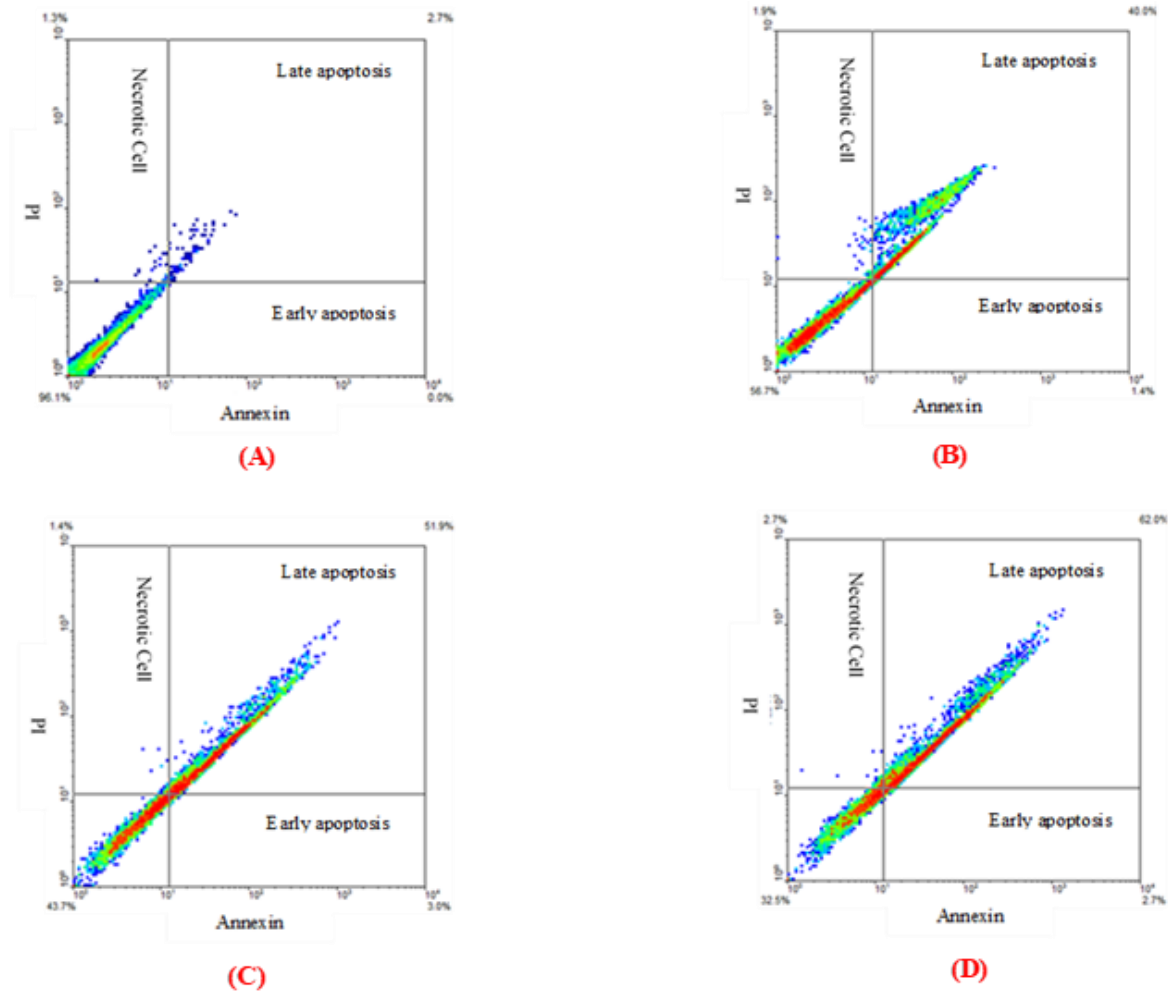


Figure 2. Flow cytometry analysis

(A) control group (control); (B) treatment with a concentration of 300  $\mu\text{g/ml}$  of *A. absinthium* extract; (C) treatment with a concentration of 12.50  $\mu\text{g/ml}$  of Taxol ; (D) Treatment with a concentration of 100 $\mu\text{g/ml}$  *A. absinthium* extract with a concentration of 10  $\mu\text{g/ml}$  Taxol

### Results of real-time PCR gene expression study:

As observed in Figure 3, BAX gene expression increased in all treatment groups in comparison to the control group. Increased BAX gene expression level in treatment groups with *A. absinthium* extract as well as the collective combination of *A. absinthium* extract with Taxol is significantly different from the control group. Expression of p53 gene was also significantly

Figure 3; the results of Annexin V-FITC test show that while in the control group about 96% of HepG2 cells are alive, in the group treated with *A. absinthium* extract about 41% of the cells suffer from apoptosis. Also, in the Taxol treatment group, the percentage of cells with apoptosis is about 54%, and we see the most cell death in the form of secondary apoptosis in the combined treatment group of *A. absinthium* extract with Taxol, so that in this group, in 62% of HepG2 cancerous cells have occurred secondary apoptosis.

increased in the combined treatment group of *A. absinthium* extract with Taxol compared to the control group. Increased expression of caspase 3 and 9 apoptotic genes in the group treated with *A. absinthium* extract was not significant compared to the control group; while, the highest increase in expression of caspase 3 and 9 genes was seen in the group treated with the combination of *A. absinthium* extract with Taxol.

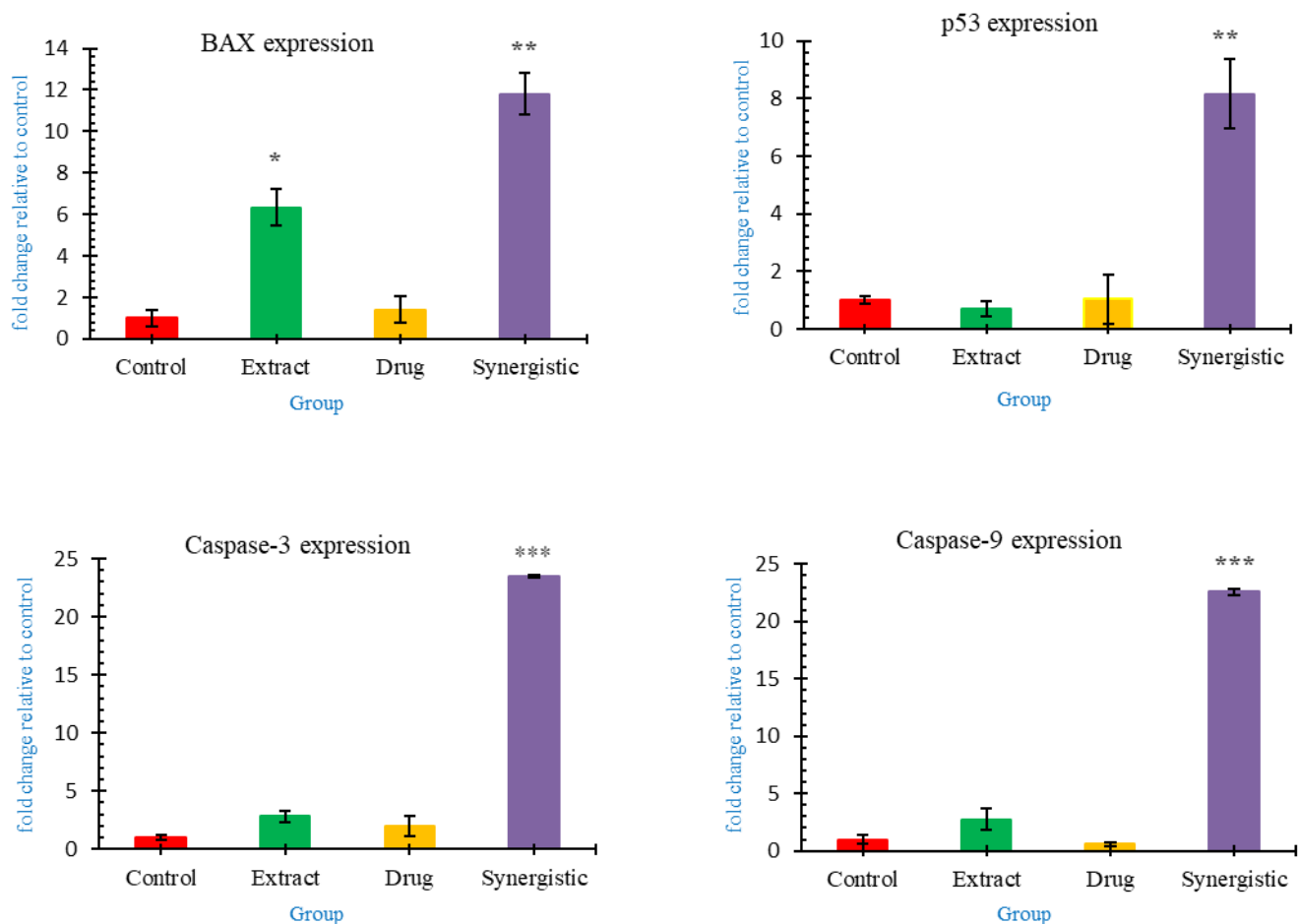


Figure 3. Evaluation of changes in the expression of apoptotic genes "BAX, Caspase 3, 9, p53" by Real-time PCR technique (p<0/05\*) (p<0/01\*\*) (p<0/001\*\*\*)

## DISCUSSION:

The cytotoxic effects of *A. absinthium* extract, Taxol, and the combination of *A. absinthium* extract and Taxol on cell proliferation were assessed in this experimental investigation using the MTT test after the methanolic extract of *A. absinthium* was prepared. Also, DAPI staining and flow cytometry analysis were used to evaluate apoptosis in cells under treatment, and finally changes in the expression of apoptotic genes "BAX, Caspase 3, 9, p53" were examined by real-time PCR technique.

The results of the MTT test showed that *A. absinthium* extract and Taxol could reduce the viability of cancerous cells of HepG2 line in a concentration-dependent manner. The best result was obtained when the combined use of *A. absinthium* extract and Taxol was used to treat the cells. In this way that cell proliferation was significantly inhibited in this treatment medium. These results indicate that the combination of *A. absinthium* extract with Taxol has a greater effect on inhibiting cell proliferation; which this matter can be demonstrated by the decrease in the concentration of "IC<sub>50</sub>" compared to when we used *A. absinthium* extract and Taxol alone.

Gordanian *et.al* (2014) investigated the cytotoxic effects of extracts of five different Artemisia species

(including *A. absinthium*) against the MCF-7 cancer line. The results of MTT test showed that the extracts of five different species of Artemisia cause to inhibit the proliferation of breast cancerous cells in a concentration-dependent manner, and also showed that the cytotoxic activity of *A. absinthium* and *A. vulgaris* species is higher than other species (30). The results from MTT test performed in this study also showed the inhibitory effects of *A. absinthium* extract in a concentration-dependent manner on the proliferation of HepG2 cancerous cells.

Kim *et al* (2018) investigated cytotoxic and anti-proliferative effects of ethanolic extract of *Artemisia capillaris* leaves against Huh7 and HepG2 cancerous cell lines and showed that *A. capillaris* extract strongly suppresses the proliferation of these cells and leads to induce apoptosis in Huh7 and HepG2 cells by increasing the expression level of caspase-3 (31); which is consistent with all the results of the present study.

Examination of morphological changes of nuclei by DAPI staining showed that morphological changes are seen in the treatment groups compared to the control group. As shown in Figure 1, in the treatment groups, the nuclei are fragmented and out of normal, which this matter could be a sign of apoptosis incidence in these cells. Most cell damage and changes in the nuclei

occur when cells are treated with a combination of *A. absinthium* extract and Taxol. The results of Annexin V-FITC test also showed that while the percentage of viability of the control group cells is about 96%; this rate decreased to 56%, 43% and 32% in cells treated with *A. absinthium* extract, Taxol and combined combination of *A. absinthium* extract with Taxol. As a result, it can be stated that the combination of *A. absinthium* extract with Taxol has a good ability to induce apoptosis in HepG2 cancerous cells.

Kim *et al* (2017) examined the ability to induce apoptosis of *Artemisia annua* extract in HCT116 cell line (colon cancer) and by using Annexin V-FITC test showed that apoptotic cells percentage increases in cells treated with different concentrations of *A. annua* extract; and also treatment with *A. annua* extract can reduce the expression of Bcl-2 anti-apoptotic gene and increase the expression of BAX apoptotic gene, and finally they concluded that *A. annua* extract has the ability to induce apoptosis in HCT116 cell line (32). All the results of laboratory experiments obtained from the present study also showed the ability of *A. absinthium* extract to induce apoptosis in the HepG2 cell line.

The results of real-time PCR to evaluate the changes rate in the expression level of "BAX, Caspase 3,9, p53" apoptotic genes showed a significant difference in increasing the expression level of these genes in the treatment group with a combination of *A. absinthium* extract and Taxol. And it can be concluded that this combination has a significant ability to increase the expression of apoptotic genes and can be given more attention in the future with more complete studies and research.

Tayarani-najaran *et.al* (2016) found cytotoxic and apoptotic effects of *Artemisia biennis* dichloromethane extract against K562 and HL-60 cell lines and showed that *A. biennis* extract causes to inhibit cancerous cell proliferation and induce apoptosis in K562 and HL-60 cells by increasing the expression level of BAX apoptotic gene (33). This is consistent with the current study's findings, which demonstrate that the extract of *A. absinthium* has the capacity to raise the expression level of apoptotic genes.

### **CONCLUSION:**

The findings obtained from this experimental research presented that the combined use of *A. absinthium* extract and Taxol significantly inhibits the proliferation of HepG2 liver cancerous cells and causes to induce cell death in these cells. Therefore, this combination can be further studied in studies related to the treatment of liver cancer.

### **Ethical Statement:**

This article does not contain any studies with human participants or animals performed by any of the authors.

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### **Conflict of Interest:**

The authors declare no conflict of interests.

### **REFERENCES:**

1. Cijo V, Dellaire G, Rupasinghe HPV. ScienceDirect Plant flavonoids in cancer chemoprevention: role in genome stability. *J Nutr Biochem*. 2017;45:1–14.
2. Dutta S, Mahalanobish S, Saha S, Ghosh S, Sil PC. Natural products: An upcoming therapeutic approach to cancer. *Food Chem Toxicol*. 2019;128(February):240–255.
3. Elsayed EA, Sharaf-Eldin MA, Wadaan M. In vitro evaluation of cytotoxic activities of essential oil from *Moringa oleifera* seeds on HeLa, HepG2, MCF-7, CACO-2 and L929 cell lines. *Asian Pacific J Cancer Prev*. 2015;16(11):4671–4675.
4. Mohammadian M, Soroush A, Mohammadian-Hafshejani A, Towhidi F, Hadadian F, Salehiniya H. The incidence and mortality of liver cancer and its relationship with development in Asia. *Asian Pacific J Cancer Prev*. 2016;17(4):2041–2047.
5. Lee J-S. Genomic Profiling of Liver Cancer. *Genomics Inform*. 2013;11(4):180185.
6. Wei Z, Doria C, Liu Y. Targeted therapies in the treatment of advanced hepatocellular carcinoma. *Clin Med Insights Oncol*. 2013;7:87–102.
7. Zheng Q, Yang H, Wei J, Tong J long, Shu Y qian. The role and mechanisms of nanoparticles to enhance radiosensitivity in hepatocellular cell. *Biomed Pharmacother*. 2013;67(7):569–575.
8. Wang TH, Lin YS, Chen Y, Yeh CT, Huang Y lin, Hsieh TH, et al. Long non-coding RNA AOC4P suppresses hepatocellular carcinoma metastasis by enhancing vimentin degradation and inhibiting epithelial-mesenchymal transition. *Oncotarget*. 2015;6(27):23342–23357.
9. Chakravarthi BVSK, Sujay R, Kuriakose GC, Karande AA, Jayabaskaran C. Inhibition of cancer cell proliferation and apoptosis-inducing activity of fungal taxol

- and its precursor baccatin III purified from endophytic *Fusarium solani*. *Cancer Cell Int.* 2013;13(1):1–11.
10. Mei M, Xie D, Zhang Y, Jin J, You F, Li Y, et al. A new  $2\alpha,5\alpha,10\beta,14\beta$ -tetraacetoxy-4(20),11-taxadiene (SIA) derivative overcomes paclitaxel resistance by inhibiting MAPK signaling and increasing paclitaxel accumulation in breast cancer cells. *PLoS One.* 2014;9(8):1–13.
  11. Baharara J, Amini E, Nikdel N, Salek-Abdollahi F. The cytotoxicity of dacarbazine potentiated by sea cucumber saponin in resistant B16F10 melanoma cells through apoptosis induction. *Avicenna J Med Biotechnol.* 2016;8(3):112–119.
  12. Goldar S, Khaniani MS, Derakhshan SM, Baradaran B. Molecular mechanisms of apoptosis and roles in cancer development and treatment. *Asian Pacific J Cancer Prev.* 2015;16(6):2129–2144.
  13. Hassan M, Watari H, Abualmaaty A, Ohba Y, Sakuragi N. Apoptosis and Molecular Targeting Therapy in Cancer. *BioMed Research International.* 2014;2014:1-24.
  14. Getahun T, Sharma V, Gupta N. The genus *Laggetera* (Asteraceae) – Ethnobotanical and Ethnopharmacological Information, Chemical Composition as well as Biological Activities of Its Essential Oils and Extracts: A Review. *Chem Biodivers.* 2019;16(8):1-23.
  15. Ziyad A, Tilaoui M, Jaafari A, Oukerrou MA, Mouse HA. More insights into the pharmacological effects of artemisinin. *Phyther Res.* 2018;32(2):216–229.
  16. Du J, Tang XL. Natural products against cancer: A comprehensive bibliometric study of the research projects, publications, patents and drugs. *J Cancer Res Ther.* 2014;10(5):27–37.
  17. Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, et al. Effective Medicinal Plant in Cancer Treatment, Part 2: Review Study. *J Evidence-Based Complement Altern Med.* 2017;22(4):982–995.
  18. Taleghani A, Emami SA, Tayarani-najaran Z. *Artemisia* a promising plant for the treatment of cancer. *Bioorg Med Chem.* 2019;28(October 2019):1-22.
  19. Martínez-Díaz RA, Ibáñez-Escribano A, Burillo J, de las Heras L, del Prado G, Agulló-Ortuño MT, et al. Trypanocidal, trichomonocidal and cytotoxic components of cultivated *Artemisia absinthium* Linnaeus (Asteraceae) essential oil. *Mem Inst Oswaldo Cruz.* 2015;110(5):693–699.
  20. Ali M, Abbasi BH, Ahmad N, Khan H, Ali GS. Strategies to enhance biologically active-secondary metabolites in cell cultures of *Artemisia*—current trends. *Crit Rev Biotechnol.* 2017;37(7):833–851.
  21. Shafi G, Hasan TN, Syed NA, Al-Hazzani AA, Alshatwi AA, Jyothi A, et al. *Artemisia absinthium* (AA): A novel potential complementary and alternative medicine for breast cancer. *Mol Biol Rep.* 2012;39(7):7373–7379.
  22. Al-Menhali A, Al-Rumaihi A, Al-Mohammed H, Al-Mazrooey H, Al-Shamlan M, Aljassim M, et al. *Thymus vulgaris* (Thyme) inhibits proliferation, adhesion, migration, and invasion of human colorectal cancer cells. *J Med Food.* 2015;18(1):54–59.
  23. Lian G, Li F, Yin Y, Chen L, Yang J. Herbal extract of *Artemisia vulgaris* (mugwort) induces antitumor effects in HCT-15 human colon cancer cells via autophagy induction, cell migration suppression and loss of mitochondrial membrane potential. *J BUON.* 2018;23(1):73–78.
  24. Paramee S, Sookkhee S, Sakonwasun C, Na Takuathung M, Mungkornasawakul P, Nimlamool W, et al. Anti-cancer effects of *Kaempferia parviflora* on ovarian cancer SKOV3 cells. *BMC Complement Altern Med.* 2018;18(1):1–13.
  25. Baharara J, Amini E, Afzali M, Nikdel N, Mostafapour A, Kerachian MA. Apoptosis inducing capacity of *holothuria arenicola* in CT26 colon carcinoma cells in vitro and in vivo. *Iran J Basic Med Sci.* 2016;19(4):358–365.
  26. Yu XJ, Sun K, Tang XH, Zhou CJ, Sun H, Yan Z, et al. Harmine combined with paclitaxel inhibits tumor proliferation and induces apoptosis through down-regulation of cyclooxygenase-2 expression in gastric cancer. *Oncol Lett.* 2016;12(2):983–988.
  27. Kumar S, Agnihotri N. Piperlongumine, a piper alkaloid targets Ras/PI3K/Akt/mTOR signaling axis to inhibit tumor cell growth and proliferation in DMH/DSS induced experimental colon



- cancer. *Biomed Pharmacother.* 2019;109(June 2018):1462–1477.
28. 28.Mousavi B, Tafvizi F, Zaker Bostanabad S. Green synthesis of silver nanoparticles using *Artemisia turcomanica* leaf extract and the study of anti-cancer effect and apoptosis induction on gastric cancer cell line (AGS). *Artif Cells, Nanomedicine Biotechnol.* 2018;46(sup1):499–510.
  29. 29.Wu L, Cheng Y, Deng J, Tao W, Ye J. Dihydroartemisinin inhibits proliferation and induces apoptosis of human hepatocellular carcinoma cell by upregulating tumor necrosis factor via JNK/NF- $\kappa$ B pathways. *Evidence-based Complement Altern Med.* 2019;1-9.
  30. 30.Gordanian B, Behbahani M, Carapetian J, Fazilati M. In vitro evaluation of cytotoxic activity of flower , leaf , stem and root extracts of five *Artemisia* species. *Res Pharm Sci.* 2014;9(2):91–96.
  31. 31.Kim J, Jung KH, Yan HH, Cheon MJ, Kang S, Jin X, et al. *Artemisia Capillaris* leaves inhibit cell proliferation and induce apoptosis in hepatocellular carcinoma. *BMC Complement Altern Med.* 2018;18(1):1–10.
  32. 32.Kim EJ, Kim GT, Kim BM, Lim EG, Kim SY, Kim YM. Apoptosis-induced effects of extract from *Artemisia annua* Linné by modulating PTEN/p53/PDK1/Akt/ signal pathways through PTEN/p53-independent manner in HCT116 colon cancer cells. *BMC Complement Altern Med.* 2017;17(1):1–12.
  33. 33.Tayarani-najaran Z, Makki F, Emami SA, Alamolhodaei N. Cytotoxic and apoptotic effects of different extracts of. *Iran J Basic Med Sci.* 2016;20(21):167–171.