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Investigating the cytotoxic effect of ibuprofen concentration in liver cancer cells (HepG2) and normal fibroblast (AGO)

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Author contributions
Abbas Zabihi was responsible for the original draft writing; Sanaz Pashapour, Abbas Zabihi and Roya Behrouzi were responsible for reviewing, editing, and supervision; Abbas Zabihi and Sanaz Pashapour was responsible for methodology.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
DMEM, Dulbecco's Modified Eagle Medium; RPMI, Roswell Park Memorial Institute; HCl, hydrochloric acid.

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Abstract
Objective: Although many studies have reported that nonsteroidal anti-inflammatory drugs can have anticancer effects, the results are still challenging. The aim of this research is to Mechanism and effect of anti-inflammatory drugs in cancer treatment. **Methods:** In this laboratory study, cell lines were randomly divided into control group (no exposure to ibuprofen and groups exposed to ibuprofen concentrations of 10, 1, 0.1, and 0.001 mg/mL. The cytotoxic effect of ibuprofen was measured at 24 and 72 hours using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Data were compared between groups using a one-way variance test. **Results:** The results showed that the viability rate of HepG2 cancer cells at concentrations of 1 and 10 mg/mL decreased significantly compared to the control group in 24 hours ($P < 0.0001$). Also, the viability rate at concentrations of 1, 10, 0.1, and 0.001 mg/mL decreased significantly compared to the control group in 72 h ($P < 0.0001$). Only the concentration of 10 mg/mL ibuprofen decreased the viability of normal cells compared to the control ($P < 0.05$). **Conclusion:** Overall, the results of this research showed that different concentrations of ibuprofen had a cytotoxic effect on liver cancer cells, and except for the concentration of 10 mg/mL, the other concentrations did not have a cytotoxic effect on normal cells.

Keywords: liver cancer cells; ibuprofen; non-steroidal anti-inflammatory drugs; cytotoxic

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Backgroubd

Liver cancer is one of the most common and malignant cancers and the main cause of death in the world and Iran [1-3]. Liver cancer is one of the most important cancers in the world, which causes the death of many people every year [4]. Liver cancer accounts for 9% of all deaths from all types of cancer. Currently, surgical removal of the tumor is the best method for this cancer and other methods are not very successful [1]. Hepatitis B and C are the main risk factors for liver cancer. In recent years, significant studies have been conducted on the apoptotic effects of non-steroidal anti-inflammatory drugs on cancer cells, but research in this field is still attractive to scientists and challenges There are many in this field. Ibuprofen is a non-steroidal anti-inflammatory drug that can be used to treat all types of cancers [5, 6]. Research findings have proven that ibuprofen is effective in treating gastrointestinal cancers [7-9]. On the contrary, some studies have shown that some non-steroidal anti-inflammatory drugs have no effect on the treatment of cancers. Despite significant findings regarding the positive effects of non-steroidal anti-inflammatory drugs in inhibiting liver cancer cells [7-9], the results of some studies in this field are disappointing [10]. On the other hand, to the best of our knowledge, there are very limited reports and definitive evidence about non-steroidal anti-inflammatory drugs in inhibiting liver cancer cells after the use of ibuprofen in the human model. Based on this and considering that common treatments are very complicated in controlling cervical cancer cells [1]. Therefore, the use of non-steroidal anti-inflammatory drugs can probably be effective in inhibiting cancer cells [7-9]. During this research, using cell culture methods, we investigated the effects of ibuprofen on liver cancer and normal cells, and the results of this research can be considered in the design of liver cancer treatment methods in human samples.

Materials and methods

According to the content of the present research, experiments based on cell culture and cell proliferation were carried out at Javed Biotechnology Research Center. Ibuprofen was obtained in the form of pure powder and in the amount of one gram from a reputable pharmaceutical company called Pharmachemy. Liver cancer cell line (HepG2) and normal human fibroblast cell line (AGO) were prepared frozen in a nitrogen tank from Pasteur Institute cell bank.

Sample preparation

First, we weighed 0.1 g of pure ibuprofen powder with a digital scale; after weighing, we poured them into a microtube, and then added 100 λ (NaOH) to it and dissolved it in sodium by Vortex. After the sample was dissolved in that medium, we added DMEM (Dulbecco's Modified Eagle Medium) culture with a concentration of 10 mg/mL. By measuring the pH, we found out that the medium has a pH of 11, which is alkaline and destroys them in contact with living cells. To solve this problem, the pH of the environment was neutralized with the help of HCl (hydrochloric acid) [11].

MTT assay

In order to measure the viability rate, first, 1×10^5 HepG2 liver cancer cells and 1×10^3 AGO normal fibroblast cells were cultured in DMEM and RPMI (Roswell Park Memorial Institute) culture medium in each 96-well well. After 24 hours, they were treated with ibuprofen concentrations of 10, 1, 0.1, and 0.001 mg/mL. After 72 and 24 hours of exposure to ibuprofen, the supernatant was removed and then 80 microliters of fresh culture medium and 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich) solution were added. After placing the plates in the incubator for 4 hours, the culture medium was completely drained and 200 µL of dimethyl sulfoxide solution was added to each well. After the purple formazan crystals were completely dissolved by the dimethyl sulfoxide solvent, the absorbance of the samples was measured at a wave length of 570 nm with an ELISA Reader device [2, 12-17].

Statistical analysis

The data were analyzed using SPSS software, the Kolmogorov-Smirnov test (for the normal distribution of data), one-way analysis of variance, and Tukey's post hoc test. Became. A significance level of $\alpha < 0.05$ was considered.

Results

Investigating the survival effects of ibuprofen on liver cancer cells (HepG2) during 24 and 72 hours. The results showed that the survival rate of HepG2 cancer cells in the treatment with ibuprofen at concentrations of 1 and 10 mg/mL was significantly decreased compared to the control group in 24 hours ($P < 0.0001$). At the concentration of 0.001 and 0.1 mg/mL ibuprofen in 24 hours, no significant difference was observed compared to the control. Also, the viability rate at concentrations of 1, 10, 0.1 and 0.001 mg/mL decreased significantly compared to the control group in 72 hours. ($P < 0.0001$) (Figure 1).

Investigating the viability effects of ibuprofen on normal cells (AGO1522) in 24 and 72 hours the results showed that the survival rate of normal AGO cells in the treatment with concentrations of 10, 1, 0.1 and 0.001 mg/mL of ibuprofen compared to the control group in 24 hours was not observed to be significantly different. In 72 hours, only the concentration of 10 mg/mL ibuprofen compared to the control caused a decrease in viability ($P < 0.05$) (Figure 2).

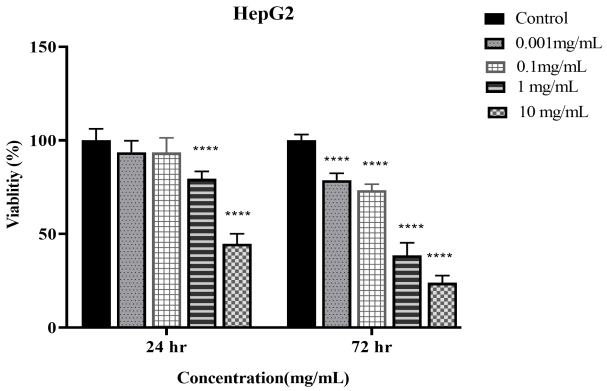

Figure 1 Viability percentage of HepG2 cancer cells after treatment with ibuprofen in 24 and 72 hours. * Indicates significance compared to the control group. (****$P < 0.0001$)

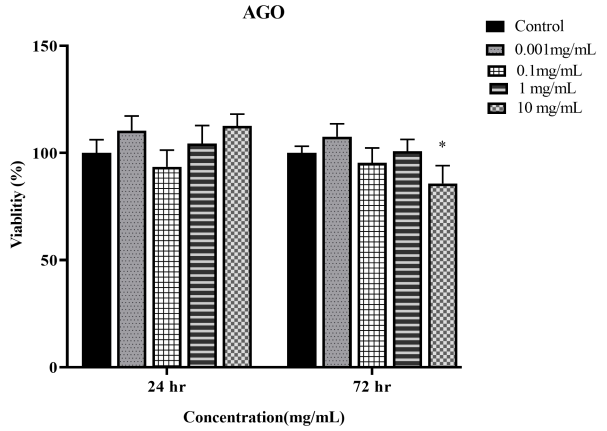

Figure 2 The survival percentage of normal AGO cells after treatment with ibuprofen in 24 and 72 hours. * Indicates significance compared to the control group. (*$P < 0.05$)

Discussion

Although many studies have shown that steroidal anti-inflammatory drugs can be used to inhibit cancer cells [2, 18, 19], the effect of ibuprofen on reducing the proliferation of cancer cells, especially liver cancer, is still one of the most challenging research topics. Based on this, the present study investigated the effect of the cytotoxic concentration of ibuprofen in liver cancer cells (HepG2) and normal fibroblast (AGO) by using the MTT assay method, to show that ibuprofen is able to inhibit the proliferation of liver cancer cells at the right time. The results showed that the viability rate of HepG2 cancer cells in the treatment with ibuprofen at concentrations of 1 and 10 mg/mL was significantly decreased in 24 hours. Also, the viability rate at concentrations of 1, 10, 0.1 and 0.001 mg/mL decreased significantly in 72 hours. In 72 hours, only the concentration of 10 mg/mL ibuprofen compared to the control caused a decrease in viability. In agreement with this finding, Ratana Lek Somboon investigated the cell viability of ibuprofen by MTT method on KKU-M139 bile cancer cells in 48 hours. The results showed that these drugs can reduce the survival of bile cancer cells in a dose-dependent manner [7]. Mamatha Nakka showed in a study that the combination of acyl hydrazones with ibuprofen can suppress human prostate cancer cells PC-3 done [20]. Research findings have shown that ibuprofen can act as a preventive agent in pancreatic cancer or a risk-reducing factor in colorectal cancer [21, 22]. In another study, Ratana Lek Somboon investigated the cytotoxic effect of ibuprofen on colon cancer cells with the MTT method, and the results of this study showed that ibuprofen can suppress cancer cells in a dose-dependent manner, and it was reported that ibuprofen may be used as an anti-proliferative agent can be used to treat colon cancer in the future [7]. In terms of the possible mechanism of the cytotoxic effect of ibuprofen on apoptosis in liver cancer cells, it can be said that according to previous studies [10, 19], ibuprofen probably exerts its effect through mitochondrial pathways by binding to the receptors of liver cancer cells [23]. However, in order to accurately investigate the effects of cytotoxic concentrations of ibuprofen on liver cancer cells, more extensive research is needed, especially in the field of expression of other apoptotic and genes. The scope of the study of this research is only in the scope of investigating the effects of cytotoxic concentration of ibuprofen in liver cancer cells in cell culture medium. The researchers of this research hope it will be possible to investigate the effects of ibuprofen on the expression of other apoptotic and genes in the future.

Conclusion

Overall, the results of this research showed that different concentrations of ibuprofen had a cytotoxic effect on liver cancer cells, and except for the concentration of 10 mg/mL, the other concentrations did not have a cytotoxic effect on normal cells.

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