



# Anti-cancer Effects of Ketoprofen on the Expression of *HE4* Gene and Viability of the A2780 Human Ovarian Cancer Cell Line

Maryam Yahyaie<sup>1</sup>, Majid Morovati-Sharifabad<sup>1,\*</sup>, Elham Salehi<sup>1</sup>, Fatemeh Sarkargar<sup>2</sup> and Gholamhosein Pourghanbari<sup>3</sup>

<sup>1</sup>Department of Basic Sciences, Faculty of Veterinary Medicine, Ardakan University, Ardakan, Iran

<sup>2</sup>Meybod Genetic Research Center, Meybod, Iran

<sup>3</sup>Department of Clinical Sciences, Faculty of Veterinary Medicine, Ardakan University, Ardakan, Iran

\*Corresponding author: Department of Basic Sciences, Faculty of Veterinary Medicine, Ardakan University, Ardakan, Iran. Email: mmorovati@ardakan.ac.ir

Received 2020 December 23; Revised 2021 January 20; Accepted 2021 January 21.

## Abstract

**Background:** Ovarian cancer is the second leading cause of death in Iran compared with other gynecological diseases. Considering the role of cyclooxygenase (COX) enzymes and prostaglandin E2 production in tumor lesions, nonsteroidal anti-inflammatory drugs (NSAIDs) show antitumor properties by inhibiting COX. Furthermore, some compounds can serve as non-selective inhibitors of COX (such as ketoprofen) and prevent cancer development. Human epididymis protein 4 (*HE4*) is one of the most sensitive tumor markers known in the study of the disease of ovarian epithelial cancer. The expression of *HE4* increases in different types of ovarian cancer.

**Objectives:** The aim of this study was to determine the anti-cancer effects of ketoprofen on the viability of ovarian cancer cells and expression of *HE4* gene.

**Methods:** To calculate half-maximal inhibitory concentration (IC<sub>50</sub>), A2780S cells were treated with different concentrations of ketoprofen for 24 hours, then the cells were incubated with appropriate concentrations of IC<sub>50</sub> for 24, 48, and 72 hours. Real-time polymerase chain reaction (PCR) was used to measure changes caused by the effect of drugs on *HE4* gene expression and analyzed by the  $2^{-\Delta\Delta CT}$  method.

**Results:** The IC<sub>50</sub> level of ketoprofen for 24 hours was 583.7  $\mu$ M. According to real-time PCR results, treatment of cells with ketoprofen reduced *HE4* expression.

**Conclusions:** *HE4* gene expression decreased in cells treated with ketoprofen compared with the cells in the control group, which proves the anti-cancer activity of ketoprofen and a reduction in the viability of ovarian cancer cells.

**Keywords:** Ovarian Cancer, A2780S Cell Line, Ketoprofen, *HE4* Gene

## 1. Background

Ovarian cancer is the eighth most common cause of cancer death due to gynecologic malignancies. And is the second cause of death cancer due to cancer in Iranian women (1). Epithelial ovarian tumors are highly common in the sixth decade of a women's life. Serous carcinoma is the most common epithelial ovarian tumor (2). Therefore, given the extensive prevalence of ovarian cancer, it is urgent to conduct further studies to detect novel anti-cancerous components with high therapeutic utility and minimum side effects.

Microscopic and macroscopic evaluations have confirmed the positive effect of ketoprofen on tumor weight loss and treatment of ovarian cancer in Wistar rats

(3). Ketoprofen is a relatively novel nonsteroidal anti-inflammatory drugs (NSAIDs). It is mainly used for relieving pain and reducing fever and arthritis-related inflammation. Previous studies have shown that NSAIDs exhibit anti-tumoral effects by inhibiting cyclooxygenase (COX) and inducing cellular death through activating caspase and stopping the cellular cycle (4). Researchers have concluded that molecules with COX-inhibiting features are potential anti-cancer agents. NSAIDs are used to inhibit the growth of cancerous cells in glioma (5), melanoma (6), ovarian cancer (7), colorectal cancer (8), prostate cancer (9), and osteosarcoma (10). One of the potential effects of NSAIDs is inhibiting the expression of the Cyclin D<sub>1</sub> enzyme, which is a regulator of the cellular cycle and activates TNF-induced NF- $\kappa$ B. Ketoprofen has been reported as

a preferred inhibitor of COX-1, and the non-selective inhibition of COX-1 and COX-2 has been revealed using ketoprofen (11). COX-1 is increasingly expressed in cancerous cells of ovaries (12), colon, lung (13), prostate (14), cervix (15), and breast (16), while they are hardly detected in normal tissues. Ketoprofen may have a role in reducing angiogenesis and tumor progression by inhibiting the absorption of platelets, neutrophils, and lymphocytes, in addition to modulating the effects of COX and lipoxygenase (LOX) (17).

In previous studies, new biomarkers for early detection of ovarian cancer, including prostasin (PRSS8), kallikrein (KLK6/7), and miRNAs, have been investigated. *HE4* is one of the most promising tumor markers for the progression of ovarian mucosal cancer, which its high sensitivity and specificity for *HE4* and high expression in serum and tissue of patients with ovarian cancer were the reasons for choosing this tumor marker (18). *HE4* is one of several WAP proteins on 20q12 - 13.1 that is expressed by WFDC2. Using molecular techniques to measure *HE4* protein is a relatively new non-invasive diagnosis method to determine the severity of disease invasion, early diagnosis of cancer, and screening the ovarian epithelial cancer patients in terms of disease relapse and progression. Concurrent evaluation of *HE4* and *CA125* in patients' serum, known as the Roma test, makes it possible to accurately detect women with ovarian cancer before menopause (19). Increased expression of *HE4* in patients' urine, serum, and ovarian neoplastic tissue has been reported. The *HE4* gene is expressed in 100% of endometrioid carcinoma, 93% of serous adenocarcinoma, and 50% of transparent cell adenoma, which helps divide the different subgroups of tumors (20).

## 2. Objectives

Considering the importance of ovarian cancer, the present study was conducted to determine the anti-cancer effect of ketoprofen on the expression of the *HE4* gene and the survival of ovarian cancer cells of A2780S.

## 3. Methods

### 3.1. Cell Culture

In this study, human ovarian cancer cell line A2780S obtained from the Pastor Institute of Iran was used. The cells were cultured in RPMI1640 medium (Inoclon; Iran), containing 10% FBS (German-made Biochrom GmbH) and 1% penicillin-streptomycin antibiotic (Inoclon Company;

Iran) and incubated in optimum conditions. Adherent cultures should be passaged when they are in the log phase after cell density reached 80 - 70%.

### 3.2. Drug

Ketoprofen solution (Erfan Darou; Iran) was prepared as 100 mg/mL from the pharmacy. For serial dilutions 1, 5, and 10  $\lambda$ , ketoprofen was dissolved in RPMI1640 medium.

### 3.3. Cellular Part

#### 3.3.1. IC50 Assay & Calculation of Time

To obtain IC50 cultures, after reaching the desired cell density, the cells in the plate were separated from the flask floor. Then, the cells were implanted in a 12-well plate. They were then incubated in optimum conditions. After reaching the desired density, different doses of ketoprofen were added to each well. After trypsinization, the cells were separated. After centrifugation of cell suspension, cell counting was done using the Neubauer slide. Then, for calculation of time, the IC50 method was performed, except that the appropriate dose obtained by the IC50 method was added to the wells, and the cells were harvested at different times of 24, 48, and 72 h.

#### 3.3.2. Cell Culture to Measure Gene Expression

The cells were implanted in 24-well plates and were incubated in optimum conditions. After reaching the desired density, a dose of 500  $\mu$ M of ketoprofen was added to the wells, and cell harvesting was performed within 24 and 48 h. After centrifugation of the cell suspension, cell deposition quickly entered the nitrogen tank. In all experiments, a control group (without receiving ketoprofen) was considered.

### 3.4. Molecular Experiments

#### 3.4.1. RNA Extraction

RNA extraction was performed using the High Pure RNA Isolation Kit (Roche Company made in Germany) according to its protocol. For qualitative evaluation of the extracted RNA, RNA electrophoresis was performed using an agarose gel. High purity RNA creates two clear bands on the gel. Also, for quantitative evaluation of the extracted RNA, a NanoDrop spectrophotometer was used, and optical absorption was measured at the wavelengths of 230, 260, and 280 nm.

### 3.4.2. cDNA Synthesis

cDNA synthesis was performed based on a thermo scientific kit made in Lithuania, which after mixing materials, was placed in a thermal cycler for 10 min at 25°C, 60 min at 42°C, and 10 min at 65°C, then a NanoDrop spectrophotometer was used to measure the absorption of cDNA samples.

### 3.4.3. Primer Design

To design primers, the sequences of *HE4* and *GAPDH* genes were obtained from NCBI databases and designed using specific primers and the Gene Runner software. After designing the sequences of the primer, the accuracy primers' coupling location was ensured using the NCBI Blast. The primers were delivered as lyophilized from Pishgam Biotech company (Table 1).

### 3.4.4. Investigation of the Gene Expression Using SYBR Green in Real-time PCR

Real-time PCR reactions were performed (Applied Biosystems StepOne™ and StepOnePlus; USA) in a final 25  $\mu$ L volume. At first, a mixture of RealQ plus 2x master mix (Ampliqon; Denmark) and water were prepared. The *HE4* and *GAPDH* primers were added to their master mix solution, and then cDNA was added. Real-time PCR was conducted, and the temperature-time program was performed in three stages. The first stage, which leads to the denaturation of the pattern DNA and activation of the polymerase enzyme, continued at 95°C for 15 minutes, and in the second stage, the DNA proliferation reaction consisted of 40 cycles at 95°C for 20 seconds, 62°C for 15 seconds, and 72°C for 30 seconds. The final step was to check the specificity of the product to plot the melting curve at the temperature range of 73 - 95°C, which was set for each degree for 5 seconds.

### 3.5. Statistical Analysis

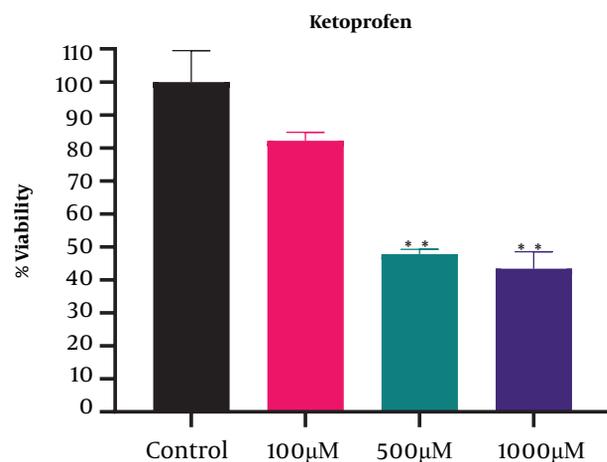
All experiments reported in the present study were performed in triplicate. The data were analyzed by the one-way analysis of variance (ANOVA) using GraphPad PRISM (Version 8), and they were reported as mean  $\pm$  standard deviation (SD). A  $P < 0.05$  was considered statistically significant.

## 4. Results

### 4.1. IC50 Assay

The results obtained after a 24-hour incubation showed a decrease in the survival rate after treatment with

ketoprofen in a dose-dependent manner (Figure 1) so that the percentage of living cells compared with the control decreased significantly from  $82.21 \pm 2.5$  (at a concentration of 100  $\mu$ M) to  $43.43 \pm 5.15$  (at a concentration of 1000  $\mu$ M). According to Figure 1, the IC50 for ketoprofen was 583.7  $\mu$ M, which was followed by treatment using a concentration of 500  $\mu$ M. According to the Tukey test results, there was no significant difference between the two doses of 500  $\mu$ M and 1000  $\mu$ M; however, there was a significant difference between these treatments and the control group at  $P < 0.05$  (Table 2).



**Figure 1.** Comparison of viability between different concentrations of ketoprofen. The diagram is drawn based on the percentage of living cells compared with the control group. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ .

### 4.2. Calculation of Time

According to the results displayed in Figure 2, significant differences were observed at different times ( $P < 0.05$ ) and the survival rate decreased over time; however, the highest lethality of ketoprofen was found 24 hours after treatment ( $55.14 \pm 5.78$ ). According to the Tukey test results, there was no significant relationship between the treatment groups; however, treatment with ketoprofen after 24, 48, and 72 hours showed significant compared with the control group at  $P < 0.05$  (Table 3).

### 4.3. HE4 Gene Expression

According to Figure 3, the *HE4* gene expression in the A2780S cell line treated with ketoprofen decreased compared with the cells in the control group (Fold change  $< 1$ ). The trend of expression reduction in cells that received ketoprofen for 48 hours (Fold change = 0.058234) was higher

**Table 1.** Sequences of the Primers Used in This Research

Primer Name	Sequence (5´ to 3´)	T <sub>m</sub>	%GC	Product Size (bp)
<b>HE4</b>				164
F	CGGCTTCACCCTAGTCTCAG	59.54	60	
R	CATTGGGCAGAGAGAGAAG	58.62	55	
<b>GAPDH</b>				102
F	TCCTCCACCTTTGACGCTG	59.63	57.89	
R	CACCACCCTGTTGCTGTAGC	61.24	60	

**Table 2.** Comparison of the Mean Viability at Different Ketoprofen Doses Using Tukey Test

Tukey Test	Mean Diff.	95.00% CI of Diff.	P-Value
100 µM vs. control	-17.79	-40.61 to 5.029	0.1064
500 µM vs. control	-52.15	-74.97 to -29.34	0.0026
1000 µM vs. control	-56.57	-79.39 to -33.76	0.0019
500 µM vs. 100 µM	-34.36	-57.18 to -11.55	0.0123
1000µM vs. 100µM	-38.78	-61.60 to -15.97	0.0079
1000 µM vs. 500 µM	-4.420	-27.24 to 18.40	0.8567

**Table 3.** Comparison of the Mean Viability at Different Times of Treatment with Ketoprofen Using Tukey Test

Tukey Test	Mean Diff.	95.00% CI of Diff	Adjusted P-Value
24h vs. control	-44.86	-70.03 to -19.69	0.0066
48h vs. control	-56.25	-81.41 to -31.08	0.0028
72h vs. control	-63.98	-89.14 to -38.81	0.0017
48h vs. 24h	-11.39	-36.55 to 13.78	0.3747
72h vs. 24h	-19.12	-44.28 to 6.054	0.1145
72h vs. 48h	-7.73	-32.90 to 17.44	0.6329

than those that received ketoprofen for 24 hours (Fold change = 0.06778), but based on the Tukey test results, the relationship between the two groups was not significant (Table 4).

## 5. Discussion

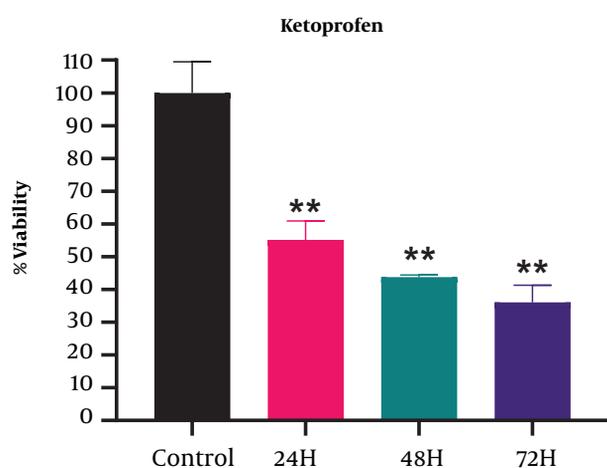
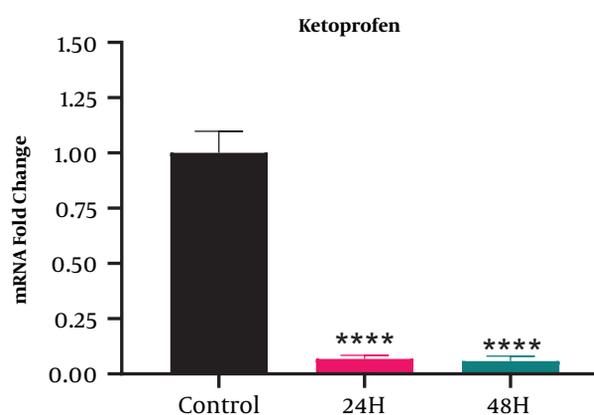
Ovarian cancer is the most important etiology of death due to gynecologic malignancies. It is curable in the initial stages but rarely in advanced stages. Using particular tumor markers with diagnostic potential is a beneficial measure in improving treatment outcomes. If ovarian cancer is not invaded out of the ovaries, it can be diagnosed and treated, and the 5-year survival reaches 95%; however, only 25% of ovarian cancers are diagnosed in the early stages (2). A2780S cell line includes adherent and epithelial-like cells. This cell line has been selected due to its optimum proliferation rate. The cells of the A2780S cell line are cisplatin-

resistant, and most ovarian cancer cells show resistance against cisplatin; thus, studies on these cells can provide information regarding cancer (21). In the present study, the effects of ketoprofen were assessed on the viability of the A2780s human ovarian cancer line.

In this study, ketoprofen was injected into the cell line at 24, 48, and 72 hours. The survival rate decreased with time, but the highest mortalities occurred after 24 hours ( $55/14 \pm 5/78$ ) (Figure 2). One of the reasons for this observation may be the presence of enough space for affecting the cells because the cell layers are increased with time and cell proliferation and decrease the effect of the drug. Another reason can be the initial shock of the drug on cells, which will cause the highest mortality. The results of the study showed a decrease in the expression of the HE4 gene and this reduction increased with time and indicated significant differences between the studied groups ( $P < 0.0001$ ) (Figure 3). This finding could be explained by the fact that

**Table 4.** Comparison of the Effect of Treatment with Ketoprofen Using Tukey Test

Tukey Test	Mean Diff.	Adjusted P-Value	Fold Change	Interpret
24h vs. control	-3.883	< 0.0001	0.06778	down: 14.754 fold
48h vs. control	4.102	< 0.0001	0.058234	down: 17.172 fold
48h vs. 24h	-0.2186	0.7809	0.859399	down: 1.164 fold

**Figure 2.** Comparison of viability at different times of ketoprofen treatment. The diagram is drawn based on the percentage of living cells 24, 48, and 72 hours after ketoprofen treatment at a dose of 500  $\mu$ M compared with the control group. \*  $P < 0.05$ , \*\* $P < 0.01$ , and  $P < 0.001$ .**Figure 3.** Comparison of the *HE4* gene expression using real-time PCR after treatment with ketoprofen for 24 and 48 hours in A2780S ovarian cancer cell line. The control group was used as the reference group to calculate fold change. \*  $P < 0.05$ , \*\* $P < 0.01$ , and  $P < 0.001$ .

ketoprofen is a non-steroid component and a non-selective inhibitor of cyclooxygenase, and considering the role of cyclooxygenase in reducing the tumor cells' angiogenesis, inhibiting the aromatase enzymes, reducing cell proliferation, and increasing apoptosis, it could be said that by inhibiting cyclooxygenase, ketoprofen reduces the tumor growth and has anti-cancer and cytotoxic effects in ovarian cancer.

Bundred and Barnes indicated that non-steroid components non-selectively inhibit COX and reduce tumor cells' angiogenesis, resulting in diminished cancer tumor growth. Moreover, they showed that cyclooxygenase is associated with epithelial growth factor (EGF). EGF increases the growth of cancer cells and is considered an important factor in tumor progression (22). Hu indicated that COX-1 and COX-2 inhibitors, such as NSAIDs increase apoptosis in tumor cells (23). Lee et al. showed that COX-1 and COX-2 are associated with inflammation in ovarian cancer; thus, in patients with ovarian cancer, increased expression of COX-2 is associated with low survival (24). Damjanovic et al., in their study, confirmed the effects of ketoprofen on cell

toxicity and growth inhibition of malignant cells in the Caco-2 and HeLa cells, particularly at moderate concentrations. Ketoprofen exerted a cytostatic effect even regarding resistance to chemotherapy. One of the antiproliferative mechanisms of this drug is the transcription factor of  $\text{NF-}\kappa\text{B}$  because  $\text{NF-}\kappa\text{B}$  was evident at low doses of ketoprofen. Given that  $\text{NF-}\kappa\text{B}$  is controlled by the modified expression of COX-2, the inhibition of this enzyme by ketoprofen is an important step in the treatment and prevention of colon and cervix cancer (25). Previous studies have accurately examined the effects of some NSAIDs in treating colon cancer, and their results indicated the importance of NSAIDs in the treatment and prevention of cancer (26). Da Silveira et al. concluded that ketoprofen-loaded nanocapsules (Keto-NCs) are an alternative for treating glioma because Keto-NC selectively reduces the survival of glioma cell line while causing no toxicity to astrocytes (27). Karamati et al. showed that ketoprofen reduced the tumor's weight and treated Wistar rats with ovarian cancer. Perhaps ketoprofen increases apoptosis, inhibits angiogenesis, reduces cell proliferation, and biologically modifies the tumor by inhibiting cyclooxygenase (3). All these results are in line with the results of our study. Ketoprofen could

increase apoptosis in previous studies, confirming its cytotoxic and anti-cancer effects.

Although the use of ketoprofen in treating various cancers has not been studied enough, it is promising to add COX-1 and COX-2 inhibitors to the common chemotherapeutic medications as an effective therapeutic strategy in preventing malignant tumors and increasing survival rate.

### 5.1. Conclusion

The results of this study showed that ketoprofen reduces *HE4* gene expression and viability of A2780S ovarian cancer cells and can affect ovarian cancer. However, the mechanism underlying these biological changes is unclear because various factors are involved in how cyclooxygenases affect living organisms. Therefore, more studies are needed to indicate the mechanism of action and how these enzymes affect living organisms in order to obtain a specific model for the treatment of patients with ovarian cancer. In the future, *HE4* is expected to be an integral part of serum screening tests for ovarian epithelial cancer and to be used to classify patients.

### Footnotes

**Authors' Contribution:** Majid Morovati, Elham Salehi, Maryam Yahyaie, Fatemeh Sarkargar, contributed to the conception and design of experimental work. Gholamhosein Pourghanbari, contributed to data and statistical analysis.

**Conflict of Interests:** No conflict or competing financial interests exist.

**Ethical Approval:** This experimental study was done on ovarian A2780s cancer cell line conducted at the Cell and Developmental Lab of the Basic Sciences Department, Faculty of Veterinary Sciences, Ardakan University (Ethics code: IR.YAZD.REC.1399.036).

**Funding/Support:** No funding, supporting by Ardakan university

### References

1. Permut-Wey J, Sellers TA. Cancer epidemiology. In: Mukesh Verma, editor. *Epidemiology of ovarian cancer*. 1 ed. Humana Press; 2009. p. 413-37.
2. Zayyan MS, Ahmed SA, Oguntayo AO, Kolawole AO, Olasinde TA. Epidemiology of ovarian cancers in Zaria, Northern Nigeria: a 10-year study. *Int J Womens Health*. 2017;**9**:855-60. doi: [10.2147/IJWH.S130340](https://doi.org/10.2147/IJWH.S130340). [PubMed: [29200893](https://pubmed.ncbi.nlm.nih.gov/29200893/)]. [PubMed Central: [PMC5702529](https://pubmed.ncbi.nlm.nih.gov/PMC5702529/)].
3. Keramati K, Habibi F, Baba Khani A, Mehranpour M. [Effect of ketoprofen on ovarian cancer induced by DMBA in female rat]. *Journal of North Khorasan University of Medical Sciences*. 2012;**3**(4):125-32. Persian. doi: [10.29252/jnkums.3.4.125](https://doi.org/10.29252/jnkums.3.4.125).
4. Hussain M, Javeed A, Ashraf M, Al-Zaubai N, Stewart A, Mukhtar MM. [Non-steroidal anti-inflammatory drugs, tumour immunity and immunotherapy]. *Pharmacol Res*. 2012;**66**(1):7-18. Persian. doi: [10.1016/j.phrs.2012.02.003](https://doi.org/10.1016/j.phrs.2012.02.003). [PubMed: [22449788](https://pubmed.ncbi.nlm.nih.gov/22449788/)].
5. Schonthal AH. Exploiting cyclooxygenase-(in)dependent properties of COX-2 inhibitors for malignant glioma therapy. *Anticancer Agents Med Chem*. 2010;**10**(6):450-61. doi: [10.2174/1871520611009060450](https://doi.org/10.2174/1871520611009060450). [PubMed: [20879982](https://pubmed.ncbi.nlm.nih.gov/20879982/)].
6. Schwartz GG, Skinner HG. Prospective studies of total and ionized serum calcium in relation to incident and fatal ovarian cancer. *Gynecol Oncol*. 2013;**129**(1):169-72. doi: [10.1016/j.ygyno.2012.12.046](https://doi.org/10.1016/j.ygyno.2012.12.046). [PubMed: [23313738](https://pubmed.ncbi.nlm.nih.gov/23313738/)].
7. Li W, Tang YX, Wan L, Cai JH, Zhang J. Effects of combining Taxol and cyclooxygenase inhibitors on the angiogenesis and apoptosis in human ovarian cancer xenografts. *Oncol Lett*. 2013;**5**(3):923-8. doi: [10.3892/ol.2012.1086](https://doi.org/10.3892/ol.2012.1086). [PubMed: [23426648](https://pubmed.ncbi.nlm.nih.gov/23426648/)]. [PubMed Central: [PMC3576189](https://pubmed.ncbi.nlm.nih.gov/PMC3576189/)].
8. Kraus S, Naumov I, Arber N. COX-2 active agents in the chemoprevention of colorectal cancer. *Recent Results Cancer Res*. 2013;**191**:95-103. doi: [10.1007/978-3-642-30331-9\\_5](https://doi.org/10.1007/978-3-642-30331-9_5). [PubMed: [22893201](https://pubmed.ncbi.nlm.nih.gov/22893201/)].
9. Del Rosso A, Saldutto P, Di Pierro ED, Masciovecchio S, Galatioto GP, Vicentini C. Impacts of antibiotic and anti-inflammatory therapy on serum prostate specific antigen in asymptomatic men: our experience. *Urologia*. 2012;**79** Suppl 19:37-40. doi: [10.5301/RU.2012.9364](https://doi.org/10.5301/RU.2012.9364). [PubMed: [22729607](https://pubmed.ncbi.nlm.nih.gov/22729607/)].
10. Kamei S, Sakayama K, Tamashiro S, Aizawa J, Miyawaki J, Miyazaki T, et al. Ketoprofen in topical formulation decreases the matrix metalloproteinase-2 expression and pulmonary metastatic incidence in nude mice with osteosarcoma. *J Orthop Res*. 2009;**27**(7):909-15. doi: [10.1002/jor.20832](https://doi.org/10.1002/jor.20832). [PubMed: [19105229](https://pubmed.ncbi.nlm.nih.gov/19105229/)].
11. Bernard S. Mechanisms of disease epilepsy. *N Engl J Med*. 2003;**349**(13):1257-66. doi: [10.1056/NEJMra022308](https://doi.org/10.1056/NEJMra022308). [PubMed: [14507951](https://pubmed.ncbi.nlm.nih.gov/14507951/)].
12. Furstenberger G, Krieg P, Muller-Decker K, Habenicht AJ. What are cyclooxygenases and lipoxygenases doing in the driver's seat of carcinogenesis? *Int J Cancer*. 2006;**119**(10):2247-54. doi: [10.1002/ijc.22153](https://doi.org/10.1002/ijc.22153). [PubMed: [16921484](https://pubmed.ncbi.nlm.nih.gov/16921484/)].
13. Sung S, Park Y, Jo JR, Jung NK, Song DK, Bae J, et al. Overexpression of cyclooxygenase-2 in NCI-H292 human alveolar epithelial carcinoma cells: roles of p38 MAPK, ERK-1/2, and PI3K/PKB signaling proteins. *J Cell Biochem*. 2011;**112**(10):3015-24. doi: [10.1002/jcb.23226](https://doi.org/10.1002/jcb.23226). [PubMed: [21678473](https://pubmed.ncbi.nlm.nih.gov/21678473/)].
14. Kim BH, Kim CI, Chang HS, Choe MS, Jung HR, Kim DY, et al. Cyclooxygenase-2 overexpression in chronic inflammation associated with benign prostatic hyperplasia: is it related to apoptosis and angiogenesis of prostate cancer? *Korean J Urol*. 2011;**52**(4):253-9. doi: [10.4111/kju.2011.52.4.253](https://doi.org/10.4111/kju.2011.52.4.253). [PubMed: [21556211](https://pubmed.ncbi.nlm.nih.gov/21556211/)]. [PubMed Central: [PMC3085617](https://pubmed.ncbi.nlm.nih.gov/PMC3085617/)].
15. Kulkarni S, Rader JS, Zhang F, Liapis H, Koki AT, Masferrer JL, et al. Cyclooxygenase-2 is overexpressed in human cervical cancer. *Clin Cancer Res*. 2001;**7**(2):429-34. [PubMed: [11234900](https://pubmed.ncbi.nlm.nih.gov/11234900/)].
16. Nie D. Cyclooxygenases and lipoxygenases in prostate and breast cancers. *Front Biosci*. 2007;**12**:1574-85. doi: [10.2741/2170](https://doi.org/10.2741/2170). [PubMed: [17127404](https://pubmed.ncbi.nlm.nih.gov/17127404/)].
17. Van Solingen RM, Rosenstein ED, Mihailescu G, Drejka ML, Kalia A, Cohen AJ, et al. Comparison of the effects of ketoprofen on platelet function in the presence and absence of aspirin. *Am J Med*. 2001;**111**(4):285-9. doi: [10.1016/s0002-9343\(01\)00838-5](https://doi.org/10.1016/s0002-9343(01)00838-5). [PubMed: [11566459](https://pubmed.ncbi.nlm.nih.gov/11566459/)].

18. Li J, Dowdy S, Tipton T, Podratz K, Lu WG, Xie X, et al. HE4 as a biomarker for ovarian and endometrial cancer management. *Expert Rev Mol Diagn.* 2009;**9**(6):555-66. doi: [10.1586/erm.09.39](https://doi.org/10.1586/erm.09.39). [PubMed: [19732003](https://pubmed.ncbi.nlm.nih.gov/19732003/)]. [PubMed Central: [PMC3273415](https://pubmed.ncbi.nlm.nih.gov/PMC3273415/)].
19. Shahi A, Moslemi E, Izadi A. [HE4 gene overexpression in ovarian cancer]. *SSU\_Journals.* 2016;**23**(12):1179-88. Persian.
20. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res.* 2005;**65**(6):2162-9. doi: [10.1158/0008-5472.CAN-04-3924](https://doi.org/10.1158/0008-5472.CAN-04-3924). [PubMed: [15781627](https://pubmed.ncbi.nlm.nih.gov/15781627/)].
21. Januchowski R, Zawierucha P, Rucinski M, Nowicki M, Zabel M. Extracellular matrix proteins expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. *Biomed Res Int.* 2014;**2014**:365867. doi: [10.1155/2014/365867](https://doi.org/10.1155/2014/365867). [PubMed: [24804215](https://pubmed.ncbi.nlm.nih.gov/24804215/)]. [PubMed Central: [PMC3996316](https://pubmed.ncbi.nlm.nih.gov/PMC3996316/)].
22. Bundred NJ, Barnes NL. Potential use of COX-2-aromatase inhibitor combinations in breast cancer. *Br J Cancer.* 2005;**93 Suppl 1**:S10-5. doi: [10.1038/sj.bjc.6602690](https://doi.org/10.1038/sj.bjc.6602690). [PubMed: [16100520](https://pubmed.ncbi.nlm.nih.gov/16100520/)]. [PubMed Central: [PMC2361689](https://pubmed.ncbi.nlm.nih.gov/PMC2361689/)].
23. Hu M, Peluffo G, Chen H, Gelman R, Schnitt S, Polyak K. Role of COX-2 in epithelial-stromal cell interactions and progression of ductal carcinoma in situ of the breast. *Proc Natl Acad Sci U S A.* 2009;**106**(9):3372-7. doi: [10.1073/pnas.0813306106](https://doi.org/10.1073/pnas.0813306106). [PubMed: [19218449](https://pubmed.ncbi.nlm.nih.gov/19218449/)]. [PubMed Central: [PMC2642666](https://pubmed.ncbi.nlm.nih.gov/PMC2642666/)].
24. Lee JY, Myung SK, Song YS. Prognostic role of cyclooxygenase-2 in epithelial ovarian cancer: a meta-analysis of observational studies. *Gynecol Oncol.* 2013;**129**(3):613-9. doi: [10.1016/j.ygyno.2013.02.011](https://doi.org/10.1016/j.ygyno.2013.02.011). [PubMed: [23422504](https://pubmed.ncbi.nlm.nih.gov/23422504/)].
25. Damjanovic I, Najman S, Stojanovic S, Stojanovic D, Veljkovic A, Kocic H, et al. Crosstalk between possible cytostatic and antiinflammatory potential of ketoprofen in the treatment of culture of colon and cervix cancer cell lines. *Bratisl Lek Listy.* 2015;**116**(4):227-32. doi: [10.4149/bll\\_2015\\_044](https://doi.org/10.4149/bll_2015_044). [PubMed: [25773949](https://pubmed.ncbi.nlm.nih.gov/25773949/)].
26. Garcia-Rodriguez LA, Huerta-Alvarez C. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. *Epidemiology.* 2001;**12**(1):88-93. doi: [10.1097/00001648-200101000-00015](https://doi.org/10.1097/00001648-200101000-00015). [PubMed: [11138826](https://pubmed.ncbi.nlm.nih.gov/11138826/)].
27. da Silveira EF, Chassot JM, Teixeira FC, Azambuja JH, Debon G, Beira FT, et al. Ketoprofen-loaded polymeric nanocapsules selectively inhibit cancer cell growth in vitro and in preclinical model of glioblastoma multiforme. *Invest New Drugs.* 2013;**31**(6):1424-35. doi: [10.1007/s10637-013-0016-y](https://doi.org/10.1007/s10637-013-0016-y). [PubMed: [24072435](https://pubmed.ncbi.nlm.nih.gov/24072435/)].