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Balancing The Expression of *K-ras* and *CD82* Genes By Magnetic Nano-oleuropein as a New Mechanism for Inhibition of AGS Gastric Cancer Cells

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A B S T R A C T

1) Background: Gastric cancer is the fourth most common cancer and the second leading cause of cancer-associated fatality in the world. During the recent decade, nanoparticles have been used widely to reduce the severity and also the treatment of cancers. The current study was performed to transfer Oleuropein into AGS cancer cells using paramagnetic nano-particles and to evaluate their effects on the cancer cell line. 2)Methods: In this research, nine concentrations of magnetic nano-oleuropein (0, 0.15, ... 333.33 and 1000 µg/mL) were applied against AGS cells in *in-vitro* condition with three replicates in the form of a completely randomized statistical design (CRD), and cell viability was investigated using MTT and Flow Cytometry assays. To determine the molecular mechanism of this effect, the relative expression of *K-ras* and *cd82* genes were investigated using the Real-time PCR assay. 3)Results: Our results for inhibition of cancer cells by different concentrations of magnetic nano-oleuropein showed that the inhibition rate of AGS cancer cells was dependent on the concentration and exposure time of the drug. The relative expression of *K-ras* oncogene decreased at the concentrations higher than 4.12 µg/mL and increased at lower concentrations (p-value <0.01). Also, the expression of *cd82* gene at the concentrations below IC50 (23.6 µg/mL) increased while at higher concentrations reduced significantly. 4)Conclusion: Balancing the expression of *K-ras* and *cd82* oncogenes through the p53 protein could be one of the molecular reasons for inhibiting the AGS cells by magnetic nano-oleuropein. Therefore, magnetic nano-oleuropein at IC50 concentration plays a key role in inhibiting gastric cancer cells by the creation of an expression balance between *K-ras* and *cd82* genes.

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Introduction

Gastric cancer is regarded as the fourth most prevalent cancer and the second leading cause of cancer-associated death, worldwide (Crew et al., 2006). Oleuropein is a valuable compound with anticancer, anti-angiogenesis, antioxidation (Hamdi et al., 2005), and analgesic (Andreadou et al.,2006) properties (figure 1).

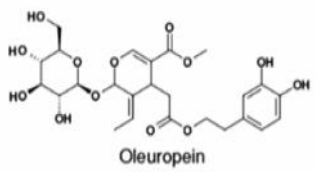

Fig. 1-Chemical Structure of Oleuropein

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Castellon et al. (Castellon et al., 2005) introduced the oleuropein as a new class of anticancer agents that inhibits several stages of cancer development. Oleuropein, as an antioxidant agent, is able to inhibit tumor development and finally by direct inhibition of cancer cells results in tumor regression. (Hamdi et al., 2005) in a study entitled "Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor" investigated the effects of Oleuropein on the proliferation of human fibroblast and human tumor cells. They determined the viable cell population using tetrazolium salt after five days and found that the growth of all cancer cells was inhibited by an increase of Oleuropein dosage. Mendez et al. (Menendez et al., 2008) in a study entitled " tabAnti-HER2 (*erb*B-2) oncogene effects of phenolic compounds directly isolated from commercial Extra-Virgin Olive Oil (EVOO)" showed that Oleuropein aglycan has strong antitumor property at micromolar concentrations, which can induce cell apoptosis in the breast cancer cells with high expression of *her2* gene. Their study revealed for the first time that all types of olive oil polyphenols remarkably affect the proliferation and survival of breast cancer cells by attenuation of the expression and activity of HER2. The findings show that as a result of olive oil consumption by humans, lignans and secoiridoids are taken by the body. In another study in 2014, it was found that the polyphenol compounds, found in olive oil and olive leaves, have strong antioxidant and anti-inflammatory properties. Currently, olive oil and olive leaves are used to treat a variety of diseases, including dermatitis, abdominal pain, injury and burn scars, hair fall, rheumatoid pains, otitis, rickets, high blood pressure, and also as a laxative and sexual stimulant agent. Also, Oleuropein, as an anti-metastasis and apoptotic agent, is used to decrease the viability of breast cancer cells (Hassan et al., 2014). In another study, Boss et al. (Boss et al., 2016) found that olive extract has anti-inflammatory and anti-cancer properties. Also, polyphenol compounds found in olive leaves were able to reduce the effects of the free radicals, produced in the body and a signal cascade caused cell stress response via the NF-κB pathway. Barbara et al. (Barbara et al., 2014) found that Oleuropein from different types of olive has an antioxidant property with good free radical scavenging potential. Also, Oleuropein showed a promising protective capability in the liver and gastric tissues.

Abtin et al. (Abtin et al., 2018) showed that the expression of *miR-155* and *Mir-21* in MCF7 cells was reduced following exposure to Oleuropein. Rakhshidan et al. (Rakhshidan et al., 2019) showed that Oleuropein was highly efficient against LNcap prostate cancer cells by altering the expression of different proteins, induction of antioxidant genes, and transcription factors, cell apoptosis induction, and inhibition of the metastatic pathways (Rakhshidan et al., 2019). Liu et al. (Liu et al., 2016) reported that Oleuropein caused inhibition of glioma U251 and A172 cells by suppression of the AKT signaling pathway. Also, Oleuropein by interaction with *Bax* and inhibition of *Bcl2* caused reduced phosphorylation of AKT. Yan Kim et al. (Yan et al., 2015) reported that Oleuropein through suppression of PI3K/AKT and generation of Reactive oxygen species (ROS) could be a promising drug for the treatment of hepatoma.

In human, the *ras* gene family is an ideal target to induce mutation in tumorigenesis related genes. It encodes a GTPase that is highly expressed in major human cells. The Ras protein is anchored in the cell membrane and acts as intercellular message transmission and is mainly involved with the activation of the epidermal growth factor (EGFR) pathway (Mao et al., 2012). It was revealed that the binding of *miR-200c to* the3'UTR region of the *K-ras* oncogene causes cancer inhibition (Song et al., 2015). Expression of *cd82/KAI1* has a diverse association with tumor development and therefore, could be considered as a good prognostic factor (Liu et al., 2006; Kauffman et al., 2003).

It may be possible that the transcription of *cd82* to be regulated directly with the p53. It is an interesting hypothesis to associate the cancer mutant genes with metastasis inhibitor genes. However, experimental studies resulted in contradictory data (Jackson et al., 2016). Several studies confirmed that the *cd82/KAI1* suppresses metastasis in several cancers, including cervical carcinoma, pancreas, ovary, lung, liver, gastric, colorectal, and breast can- cers (Raggatt et al., 2010). Thus, the increase of the *cd82/KAI1* expression may cause inhibition of

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cancer development. Nama- rata et al. (Namrata et al., 2016) showed that the expression of *cd82* and p53 in squamous cell carcinoma can be regarded as a good marker for diagnosis and possibly treatment of cancers in the future. Hinoda et al. (Hinoda et al., 1998) in 1998 reported that the attenuation of the *cd82* gene in MKN74 and NUGC3 gastric cell lines. The CD82 protein interacts with several epidermal growth factors, chemokines, and integrins which affect intracellular interactions, cell mobility, and cellular signal transmission (Tang et al., 2015). Shandiz et al. (Shandiz et al., 2016) evaluated the effect of Imatinib on the *cd82/KAI1* in gastric cancer cells and found that the expression of this gene significantly increased in the Imanitib treated cells compared with the control cells. Nanoparticles could be employed for both tumor diagnosis and drug delivery purposes (Bazak et al., 2015). Nanotechnology could result in fundamental changes in cancer treatments and care approaches (Sattler et al., 2017). The current study aimed to evaluate the anti-cancer potential of the synthesized magnetic oleuropein nanoparticles (Barzegar et al., 2019) on the expression of *K-ras* and *cd82* genes in AGS gastric cancer cells as their default expression balancing mechanism for inhibition of cancer cells.

Materials and Methods

Cell culture

AGS cancer cell line (ATCC® CRL1739™) was provided from the Pasteur Institute, Iran, and was cultured in RPMI 1640 medium, supplemented with 1% penicillin/streptomycin, and 10% fetal bovine serum (FBS). The cells were incubated at 24 °C, with 5% CO_2, and 95% humidity for 24 h.

Cell viability assay (MTT assay)

MTT assay was used to determine the cell viability using3-4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide. At first, the AGS cells were cultured in a 96 well plate with an initial population of 8×10^3 cells/well and incubated at 24 °C, with 5% CO_2, and 95% humidity for 24 h. After cell propagation, magnetic nano-oleuropein with pure Oleuropein content of 9.8% was provided and the cells were exposed to different concentrations of magnetic nano-oleuropein including, 0, 0.15, 0.45, 1.37, 4.12, 12.35, 37.04, 111.11, 333.33, and 1000 µg/mL to determine 50% inhibition concentration (IC50). After incubation for 24, 48, and 72 h, the cells were incubated with MTT solution for 4 h. Then, the supernatant was removed and the resulting formazan crystals were solubilized in dimethyl sulfoxide (DMSO). Finally, the optical density of each well was measured at 570 nm using a plate reader (Biotech, USA).

Flow Cytometry Assay

Flow cytometry assay was performed to determine the apoptosis rate among magnetic nano-oleuropein treated and control cells. The cells were treated with magnetic oleuropein, at 12 µg/mL (IC50 concentration) for 24, 48, and 72 h. then, cell staining was performed by an Apoptosis detection kit (Roch, Germany) using Annexin-V and Propidium iodide (PI).

RNA Extraction and cDNA Synthesis

RNA extraction from magnetic nano-oleuropein treated cells was performed using a TRIzol reagent (USA) according to the manufacturer's protocol. The quality and quantity of the extracted RNA were evaluated using a Nanodrop spectrophotometer (Thermo Scientific) by calculation of the A_{260}/A_{280} index. Synthesis of cDNA was performed by the Rastin Gen cDNA synthesis kit (Iran) using an oligo dT primer.

Primers

In this study, the relative expression of *K-ras* and *cd82* genes was investigated using gene-specific primers and the *GAPDH* gene was also used as an internal reference gene. The sequence of the exon regions from the mentioned genes was selected from NCBI GenBank and Ensemble databases and the gene-specific primers were designed using the Primer3 software. The designed primers were analyzed for gene specificity. The sequence of the primers used in this study was provided in Table 1.

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Table 1. Primers Used in This Study

Genes	Primer	Sequence
K-ras	F	GTGGTAGTTGGAGCTTGTGG
	R	TGACCTGCTGTGTCGAGAAT
Cd82/KAI1	F	CTCAGCCTTGTATCAAAGTCA
	R	CCCACGCCGATGAAGACATA
GAPDH₂	F	AGGGCTGCTTTTAACTCTGGT
	R	CCCCACTTGATTTTGGAGGGA

Relative gene expression

Expression of the genes was performed by a quantitative real-time PCR (qRT-PCR) kit (SinaClone, Iran) using the SYBR Green in a final reaction volume of 20 µL. The assay was performed in triplicates and after determination of the Ct value of each gene, the relative expression of them was determined by the LinReg software using the $e^{-\Delta\Delta ct}$ Method as follows:

Relative expression gene= $e^{-\Delta\Delta ct}$

$\Delta ct_1 = ct_{Target} - ct_{Hous\ Keeping\ gene}$

$\Delta ct_2 = ct_{Target} - ct_{Hous\ Keeping\ gene}$

Statistical analyses

Statistical analyses were performed by the SPSS software. ANOVA and Duncan tests were performed to determine the significant difference in cell viability between the treatments and the control ($p \leq 0.01$). Also, ANOVA was used to analyze the relative gene expression in AGS cells ($p \leq 0.05$).

Results

MTT assay

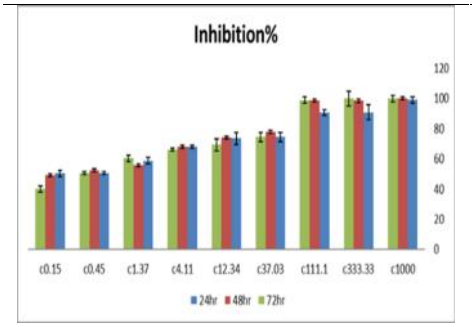
MTT assay was performed to determine the inhibition of AGS cells by different concentrations of magnetic nano-oleuropein, including 0, 0.15, 0.45, 1.37, 4.12, 12.35, 37.04, 111.11, 333.33, and 1000 µg/mL, and the results were analyzed using ANOVA test. The results showed a significant difference in cell inhibition by different concentrations of magnetic nano-oleuropein ($p < 0.01$). The results were presented in Table 2.

Table 2: Analysis of Variance for the Inhibition of AGS Cells by Different Concentrations of Magnetic Nano-oleuropein Using MTT Assay and after 24, 48 and 72 h of Incubation.

S.O.V	DF	Means of Square (MS)		
		After 24 h	After 48 h	After 72 h
Concentration	9	0.133 **	0.515 **	0.351 **
Error	18	0.03	0.05	0.159

** Significant difference at the 0.01 level of probability.

The inhibitory effect of magnetic nano - oleuropein on AGS cells during 24 h showed that the nanoparticles have an inhibitory effect even at very low concentrations. The IC50 values of the magnetic nano-oleuropein after 24, 48, and 72 exposures were determined 23.6, 15.2, and 9.2µg/mL, respectively. The results were provided in Figure 2. The results showed that the inhibitory potential of the magnetic nano-oleuropein against AGS cells was a concentration-dependent characteristic which was enhanced with an increase in nanoparticle dosage.



(a) inhibition rate of AGS cells by different concentrations of Oleuropein
Horizontal axe: Concentration (µg/mL), Vertical axe: Viability rate

(b)

LC50 $_{24h}$= 23/6

LC50 $_{48h}$= 15/2

LC50 $_{72h}$= 9/2

Fig. 2- (a) Inhibition of AGS Cells by Different Concentrations of Nano Oleuropein. (b) The IC50 Value of the Magnetic Nano-oleuropein after 24, 48 and, 72 h of Incubation.

Apoptosis/necrosis of AGS Cells

Flow cytometry assay was used to determine the apoptosis rate of magnetic nano-oleuropein treated cells. The results were provided in a two-dimensional diagram with Q1-Q4 regions, where, Q1 region represents cell necrosis, Q2,

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represents cell apoptosis, Q3, for late apoptosis, and Q4 for healthy cells (Fig. 3). The fluorochromes used in the assay include Annexin-V conjugated Fluorescein isothiocyanate (FITC) and phycoerythrin-conjugated propidium iodide (PI). To determine the effect of magnetic nano-oleuropein on apoptosis and necrosis induction in AGS cells, the percentage of the cells gated in each region was determined by the FLOWJO 7.6.1 software (Table.3). Necrosis rate (Q1 region) induced by magnetic nano-oleuropein was dependent on the exposure time that the necrosis rate increased from 7.59 to 15.8 % (about two folds) after 24 to 72 h of exposure ($p < 0.05$). Cell apoptosis is a valuable index for assessment of the inhibitory potential of different anti-cancer agents. The results showed an increasing trend for cell apoptosis during a 24-72 h of exposure to the magnetic nano-oleuropein (Q2), which increased from 4.55% to 8.67%. Evaluation of the late apoptosis induction in nano Oleuropein treated cells (Q4) showed that this characteristic was also dependent on the exposure time. However, the cells were most affected from 48 to 72 h post-exposure.

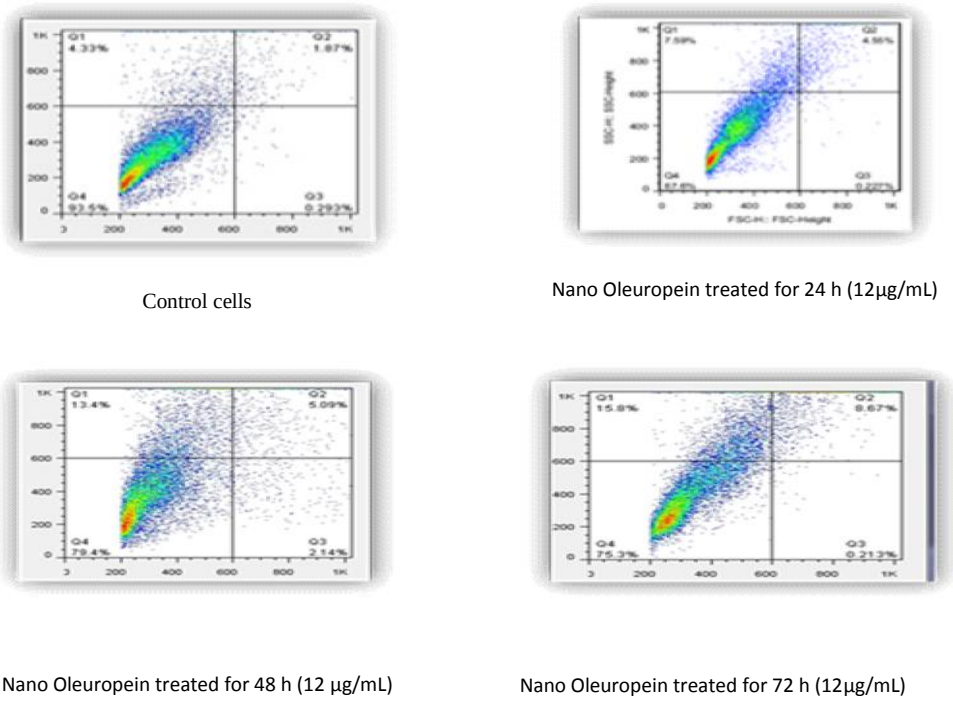

Fig. 3- Flow Cytometry Assay to Determine Cell Apoptosis/necrosis

Table 3: Percentages of Different Cells after Treatment with Nano Oleuropein

region	control	Magnetic nano -oleuropein(12µg/mL)		
		24h	48h	72h
Q1	4.33%	7.59 %	13.4 %	15.8 %
Q2	1.87%	4.55 %	5.09 %	8.67 %
Q3	0.293%	0.227%	2.14 %	0.213 %
Q4	93.5%	87.6 %	79.4 %	75.3 %

Q1: cell necrosis, Q2: cell apoptosis, Q3: late apoptosis, Q4: healthy cells after 24, 48, and 72 h of exposure.

Relative gene expression

Relative expression of *K-ras* and *cd82* genes were presented in Figure 4a and b. The S shape curves show proper amplification of the studied genes and the Ct value for the *K-ras* and *cd82* genes were in a range of 25-33 and 23-26, respectively. The sharp melting curves show specific amplification of the mentioned genes (Fig. 4: c and d).

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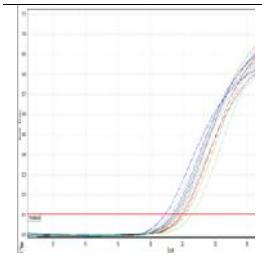
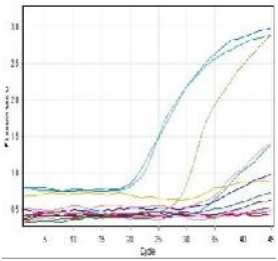
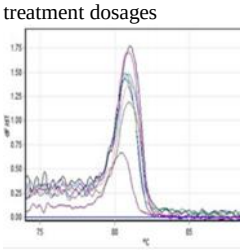


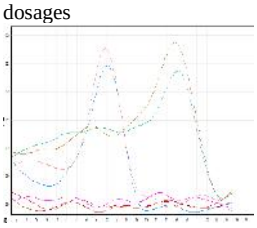
(a) amplification of the *cd82* gene at different treatment dosages



(b) amplification of the *K-ras* gene at different treatment dosages



(c) melting curve *cd82 gene*



(d) melting curves of the K-*ras (first) and GAPDH (second) genes.* The horizontal axis represents fluorescent illumination and the vertical axis represents temperature (°C).

Fig. 4- Amplification and Melting Curves of *K-ras* and *cd82 Genes*

Statistical analysis of the expression of *K-ras* and *cd82* genes in magnetic nano-oleuropein treated cells showed that the expression of the mentioned genes was significantly different between the cells treated with different concentrations of magnetic nano-oleuropein (Table. 4).

Table 4. Analysis of Variance for the Expression of *K-ras* and *cd82* gene after Exposure to Different Concentrations of Nano -Oleuropein

S.O.V	DF	MS (Means Square) *KRAS*	MS (Means Square) *cd82*
Concentration of Nano-Oleuropein (T)	9	32.81 **	8.12**
Error (E)	18	10.08	2.98

** Significant difference at the 0.01 level of probability.

Based on the result from the Duncan test, to compare the mean gene expression of *K-ras* and *cd82* genes in the cells exposed to different concentrations of magnetic nano-oleuropein, a significant difference between different treatments was observed. The results were provided in Table 5. The highest expression of the *K-ras* gene was observed in the cells treated with magnetic nano-oleuropein at concentrations of 0.15, 0.45, and 1.37µg/mL. The highest induction of the *cd82* gene was recorded in the cells treated with the nanoparticles at 37.04 µg/mL (10.87 folds), and the lowest induction for this gene was observed at 0.15 µg/mL of magnetic nano-oleuropein. According to the results, magnetic nano-oleuropein at concentrations up to 0.37 µg/mL caused the higher expression of the *cd82* gene, while at higher concentrations resulted in the attenuation of this gene. In other words, at the concentration close to IC50, the nanoparticles induced the expression of the *cd82* gene, and treatment with higher concentrations of magnetic nano-oleuropein inhibited the expression of *cd82* (Table 5). The results revealed that the magnetic nano-oleuropein could inhibit AGS cancer cells by induction of the metastasis inhibitor gene *cd82*.

Table 5. Relative Expression of *K-ras* and *cd82* Genes in AGS Cells Treated with Different Concentrations of Nano-oleuropein

Nano Oleuropein concentration (µg/mL)	Means Relative *K-ras* Expression	Means Relative *cd82* Expression
1000	0.41 ^{c}	١.٥٤ ^{b}
333.33	0.43 ^{c}	١.٧٦ ^{b}
111.11	0.73 ^{c}	١.٧٦ ^{b}
37.04	0.42 ^{c}	١٠.٨٧ ^{a}
12.35	1.95 ^{b}	١.٧٦ ^{b}
4.12	0.14 ^{c}	1.54 ^{b}
1.37	20.8 ^{a}	1.32 ^{b}
0.45	20.6 ^{a}	1.54 ^{b}
0.15	21.6 ^{a}	1 ^{b}

Different letters show a significant difference ($p < 0.01$) in the Duncan test.

Discussion

Oleuropein is a phenolic compound with anti-cancer, anti-oxidant, antibacterial, and anti-inflammatory characteristics (Hinoda et al., 1998) and its consumption with Glucose caused higher inhibitory potential against cancer cells (Hamdi et al., 2005). Previous researches showed the anti-cancer potential of several

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nanoparticles, including the cis-platin, Iron oxide, curcumin, and Anthocyanin, which were in accordance with our results (Hosseinian et al., 2015; Choudhuri et al., 2005). Wang et al., 2017, in a study on lung cancer cells, found that magnetic Iron Oxide nanoparticles caused inhibition of cancer cells by apoptosis induction through increased activity of caspase 3.

In a study performed by (Morovati et al .,2019) they evaluated the effect of simultaneous exposure of breast cancer cells to cisplatin and magnetic Iran Oxide nanoparticles. Their results showed that Iron oxide had a synergistic effect with cisplatin in cancer cell inhibition. The increased anticancer activity caused by Iron Oxide nanoparticles was in accordance with the present work. Liu et al. [Liu et al., 2018] reported that the loading of Oxaliplatin and Herceptin on Iron Oxide nanoparticles caused more efficient drug delivery to the gastric cancer cells. In this work, magnetic nano- oleuropein inhibited AGS cells after 24, 48, and 72 h of incubation, and the inhibitory potential significantly increased following longer incubation time ($p < 0.01$). In other words, the inhibitory potential of magnetic nano-oleuropein against AGS cancer cells was dependent on the exposure time and nano-particle concentration. The LC50 of magnetic nano-oleuropein after 24, 48, and 72 h of incubation were 23.6, 15.2, and 9.2µg/mL, respectively indicating the efficient inhibitory potential of the magnetic nano-oleuropein at low concentration and during the shorter exposure time. Metastasis of cancer cells is a frequent complication in cancer patients and is associated with up to 70 % of cancer-related death. Therefore, many researches focused on the expression of the genes associated with metastasis. *KAI1/cd82* suppresses cancer cell metastasis in different cancer types, including ovary, pancreas, lung, breast cancers as well as cervical carcinoma (Jackson et al., 2016). Thus, the higher expression of *KAI1/cd82* might result in metastasis inhibition.

The *KAI1/cd82* was initially found as a tumor suppressor in prostate cancer, and then, was considered as a suppressor of different tumor types. In a previous research in 2016 (Shandiz et al., 2016), the effect of Imatinib drug on the expression of *KAI1/cd82* and apoptosis in the AGS gastric cancer cell line was investigated and showed an increased dose-responsive expression of this gene. Also, the drug caused higher apoptosis induction in cancer cells. Similarly, in this study, we found a higher expression of the *cd82* gene and higher apoptosis in AGS cells.

Also, our results were similar to those from Kebriaezadeh et al. (Kebriaezadeh et al., 2016), who reported the higher expression of the *KAI1/cd82* gene and apoptosis induction in breast cancer cells by Dendrimer. In another study in 2016 (Salehi et al., 2016), the effect of silver nanoparticles synthesized using *Artemisia marschalliana* extract on the expression of *KAI1* and apoptosis induction in gastric cancer cells was investigated. Similar to our results the silver nanoparticles caused the higher expression of the metastasis inhibitor gene and induced apoptosis. Therefore, this gene could be a promising candidate for inhibition of cancer development and treatment of gastric cancer. In a research by (Luo et al.,2017) it was indicated that folic acid conjugated Iron oxide nanoparticles are a good candidate for the MRI and proper delivery of PD-L1 siRNA to the gastric cancer cells. Regarding the results from (Luo et al., 2017) the improved delivery and efficiency of Oleuropein using magnetic Iron oxide nanoparticles, is confirmed (Wu et al., 2017).

Our study showed that the expression of the *K-ras* gene in AGS cells treated with magnetic nano Oleuropein at the concentrations up to1.37 µg/mL significantly increased, while the gene was attenuated at higher concentrations of magnetic nano-oleuropein. This finding indicates that at higher concentrations inhibition of this oncogene as a mechanism for cancer inhibition occurs.

The molecular mechanism of cancer inhibition by magnetic nano-oleuropein was investigated by measuring the expression of the *K-ras* gene. At 0.15 µg/mL of magnetic nano-oleuropein, the expression of the *K-ras* was increased by 21.69 folds, compared with untreated cells. Despite the inhibition of cancer cells by nano Oleuropein, the induction of the *K-ras* gene was against our expectation. In fact, we expected that the cells have more initial proliferation, while they were initially restrained. This observation might be associated with post-transcriptional regulation of the genes.

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Increased expression of the *K-ras* gene could be considered as the second possible mechanism of cancer cell inhibition by magnetic nano-oleuropein. Increased expression of the *K-ras* gene and the encoded protein could causes chromosome instability in cancer cells (Maleki et al., 2017) and results in DNA degradation and induced cell apoptosis. Increased transcription of the *K-ras* gene and inhibition of the resulting mRNA by *miR-200* could be considered as another possible mechanism of this issue. Increased expression of *miR-200* could cause degradation of *K-ras* mRNA which results in the inhibition of cell proliferation. In fact, the *miR-200* binds to the 3'UTR region of the *K-ras* and causes mRNA degradation and cancer cell inhibition (Song et al., 2015).

The expression of the *cd82* gene at concentrations up to 37 µg/mL was increasing, while at higher concentrations showed a decreasing trend. As mentioned above, the *cd82* encodes a metastasis inhibitor membrane glycoprotein which is attenuated in tumor tissues and is induced by the p53 protein. Interpretation of the intergenic association pathways involved with cancer metastasis and apoptosis (figure 5) shows the interaction of the CD82, as a functional protein, with the *K-ras* gene through p53 protein. Overexpression of the *K-ras* gene results in the overexpression of the *p53* gene. Since the p53 is modulated by the CD82 and the p53 acts as a tumor suppressor, so the balancing between the *K-ras* and *cd82* genes could be involved with tumor development or inhibition through oncogenes or tumor suppressors. Considering the fact that preparation of the Tetraspanins-KAI1/cd82 complex directly induce EGF in epidermal cells and inhibit EFG in non-epidermal tissues, the location of the cell in which the *cd82* expression occurs, could be directly associated with the growth factor and finally the proliferation of cancer cells.

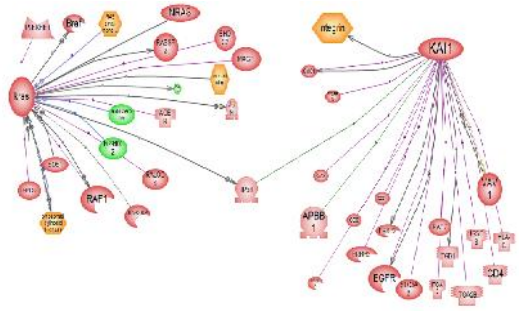

Fig. 5- Intergenic Association Pathways of the *K-ras* and *cd82* Genes and Their Interaction with Other Small Molecules

This study showed that magnetic nano - oleuropein caused apoptosis and necrosis in AGS gastric cancer cells in a dose and exposure time responsive manner. The results from the qRT-PCR assay revealed that: the expression of the *K-ras* gene at concentrations higher than 4.11µg/mL was down-regulated, while overexpressed at the lower concentration of nano Oleuropein. In contrast, the *cd82i* gene showed an increased expression at sub-IC50 concentrations and vice versa at higher concentrations. Analyzing our results shows that balancing the expression of *K-ras* and *cd82* genes by p53 protein is a key factor for the inhibition of cancer cell proliferation. Magnetic nano-oleuropein, as a natural antioxidant phenolic compound could be employed for the prevention and treatment of gastric cancers. However, this finding must be confirmed by further characterization in *in-vivo* conditions.

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