RESEARCH PAPER

Fisetin-metformin co-loaded in mesoporous silica nanoparticles (MSNs) inhibited triple negative breast cancer proliferation

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ABSTRACT

Objective(s): Herbal compounds with cytotoxicity have been of great interest in recent years to improve cancer treatment methods. Fisetin is an anti-cancer herbal compound with low solubility in aqueous systems. Metformin is another compound with anti-cancer effects. In this study, the combined effect of fisetin and metformin was investigated using mesoporous silica nanoparticles (MSNs) in breast cancer cell lines.
Materials and Methods: After the synthesis of nanoparticles, they were characterized using XRD, TEM, SEM. The DLS test showed a size of 143.4 nm with zeta-potential -39.1 mV. Fisetin and metformin were loaded into nanoparticles and loading was confirmed by FTIR. The toxicity of different concentrations of free drug (metformin, fisetin, fisetin-metformin) and Nanoformulations (metformin, fisetin and nano-fisetin-metformin) was investigated on two breast cancer lines MCF7 and MDA-MB-231.
Results: Fisetin-metformin co-loaded in MSNs showed the highest cytotoxicity among all formulations in both cell lines. The inhibition of colony formation and migration rate was effectively observed in the co-treatment of cells with fisetin and metformin loaded in nanoparticles compared to single treatments. The expression of tumor suppressor miR-200b-3p and miR-34a-5p showed that fisetin increased the expression of these tumor suppressors compared to the control.
Conclusion: The anti-cancer effect of fisetin-metformin in combination increased the expression of tumor suppressors due to the regulation of a wide range of gene network involving in cancer progress. The obtained results highlight the use of MSN as an effective drug delivery system for simultaneous delivery of herbal cytotoxic compounds in cancer.

Keywords: Breast cancer, Drug delivery systems, Flavonols, Metformin, Nanoparticles

INTRODUCTION

Ttriple-negative breast cancer (TNBC) is the most aggressive breast tumor subtype, accounts for 15% to 20% of all breast cancers [1]. It is characterized by negative expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor-2 (HER2) [2]. This type of cancer is resistant to endocrine therapy, as a result, chemotherapy is the most common systemic treatment but traditional postoperative adjuvant chemoradiotherapy is ineffective and research continues in various fields

to improve cancer treatment methods [3, 4]. Herbal medications have become increasingly important in treating cancer while reducing adverse effects [5]. Some Chinese herbal medications, when used as an adjuvant treatment with chemo- or radiotherapy reduce their side effects, improve chemo- and radiotherapeutic sensitivity while increasing the immune system function [6]. According to clinical practice, combination therapy is the future modality for treating lethal cancers with varied complexity in nature, because it has the potential to improve therapeutic efficacy [7-10]. To the best of our knowledge, combination therapy of fisetin and metformin in breast cell lines, has not been reported so far.

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Fisetin is a bioactive flavonoid, found in a variety of fruits and vegetables, including strawberries, apples, persimmons, grapes, onions, and cucumbers [11]. Fisetin has been shown to have a numerous pharmacological properties, including anti-oxidant activity, anti-inflammatory, anti-angiogenesis, and anti-tumor features [12]. According to several studies, fisetin inhibited tumor progression by suppressing cancer cell growth, invasion, migration and autophagy, as well as promoting cell cycle arrest and apoptosis [13-16]. More crucially, there is clear evidence that fisetin anti-proliferative and pro-apoptotic effects are targeted particularly in tumor cells, with normal cells being far less sensitive [17]. Metformin, a first-line treatment for type 2 diabetes, has recently been identified as a possible anti-cancer agent due to its broad pharmacological action and low toxicity [18]. Metformin has been proven to protect against breast, colon, pancreas, prostate, and liver cancer in some population studies [19]. Metformin therapy can enhance survival and lower the risk of breast cancer [20].

The use of fisetin in therapy is limited due to its low solubility in aqueous solvents. Therefore, the design of a carrier system that incorporates a large amount of drugs and specifically targets tumor cells is essential for cancer treatment (82). Different mechanisms work best for drug delivery, and nanoparticles (NP) are one of them [21]. NP-based drug delivery systems have been developed to deliver therapeutic drugs directly to solid tumors in order to improve anticancer therapy efficacy while reducing systemic toxicity [22]. Mesoporous silica nanoparticles (MSNs) due to their highly regular internal porosity (typically 2-6 nm) with large pore volume, wide surface area (700-1000 g/m3), pore size and shape adjustability and ease of structural surface changes are ideal for drug delivery system design. In addition, the degradability of MSNs facilitates the movement of these systems towards clinical application [23, 24]. MSNs with a size of about 50 to 300 nm can accumulate in the tumor microenvitonment. This preferential distribution is due to the increased effect of permeation and retention or passive targeting [25]. The molecular mechanism of fisetin effect in cancer cells is largely unknown and requires further studies. Fisetin effect has been reported on the expression of several genes involved in cancer, but the effect on epigenetics and miRNAs expression changes in breast cancer remains largely unknown. Epigenetic factors such as miRNAs play a key role in the development and treatment of cancer [26, 27]. miRNAs, small

single-stranded non-coding RNAs, are one of the post-transcriptional gene expression regulation mechanisms in eukaryotes, which after binding specifically to the target gene by inhibiting its translation cause gene expression to be silenced [28, 29].

In this study, the effect of fisetin and the combination therapy of fisetin and metformin using a MSNs delivery system on survival, colony formation, migration and the expression of tumor suppressors miR-200b-3p and miR-34a-5p in MDA-MB-231 triple negative breast cancer cells and MCF7 ER positive breast cancer cells was investigated.

MATERIALS AND METHODS
Materials

Cetyl-trimethyl ammonium chloride (CTAB), NaOH, tetraethyl orthosilicate (TEOS), metformin and fisetin were procured from Sigma-Aldrich (Deisenhofen, Germany). RPMI medium, penicillin–streptomycin, fetal bovine serum (FBS) and trypsin/EDTA were purchased from Bildia Corporation (Iran, Tehran). All other reagents and solvents were of analytical grade and have been provided by Merck company. Human breast cancer cell lines MD-AMB231 and MCF7 were provided by the Pasteur Institute of Iran.

The synthesis of MSNs

500 mg of CTAB with 1750 µl of NaOH was dissolved in deionized water in a flat bottom balloon and placed on a magnetic stirrer at stirring speed 1000 RPM until the temperature reaches 80° C. Subsequently, 2.5 mL of TEOS solution was added dropwise to obtain a milky solution. The solution was left on the stirrer for 2 hr. After the desired time and centrifugation, the obtained white precipitate was washed with ethanol and distilled water twice [30]. In order to remove surfactant MSNs were placed in an oven at 600 ° C for 6 hr.

Nanoparticles characterizations

The size and zeta potential of MSN nanoparticles were measured with dynamic laser light scattering and zeta size analyzer using Malvern Instrument (ZEN3600, UK). The pore features of the MSNs were measured by determining the nitrogen adsorption-desorption isotherms using a surface area and pore size analyzer (BELSORP MINI II, Japan). Surface analysis of the nanoparticles was calculated using the Brunauer–Emmett–Teller (BET) method and the pore size distribution was calculated by the Barrett–Joyner–Halenda (BJH) method from adsorption branches of isotherms.

XRD patterns of nanoparticles were taken by a powder X-ray diffractometer (STOE, Germany) at the wide-angle range of 2θ from 1 to 10 degrees at increments of 0.02-degree. The morphology and particle size of mesoporous silica was investigated by transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

MSN co-loaded with fisetin and metformin

50 mg (half the weight of nanoparticles) of metformin and fisetin were dissolved separately in 2000 μl of each solvent (dH_2O for metformin and 96% ethanol for fisetin) and added to the nanoparticles. Then they were placed on a magnetic stirrer for 24 hr in the dark. For the combination loading, 25 mg of metformin and fisetin were dissolved separately in the drug solvent, and they were simultaneously added to the nanoparticles suspension while stirring. After 24 hr, the solution was centrifuged, the supernatant containing unloaded free drugs was evaluated by spectrophotometric analysis to measure the efficiency of drug loading. The drug loaded nanoparticles were freeze dried and powdered for later use. Drug loading efficiency was obtained using the following formula [31].

$$\text{Loading efficiency} = \frac{(\text{primary drug} - \text{unloaded drug})\%}{\text{primary drug}}$$

The resulting absorption is substituted in the equation $y = mx + b$ to obtain the free drug concentration. By substituting it in the above formula, the efficiency of the drug loaded in the nanoparticle was calculated.

Drug release study of fisetin and metformin

Drug release from nanoparticles is the main determinant of its biological effect. Therefore, 40 mg of the drug-loaded nanoparticles were poured into the dialysis bag with PBS under neutral and acidic conditions (pH = 5.4 and pH = 7.4). The dialysis bag was placed in a shaker incubator (temperature 37°C and 100 rpm). Buffer sampling was performed at intervals of 0.5, 1, 2, 3, 6, 18, 24, 48 and 72 hr. At each time interval, 1 ml of the sample was removed and replaced with fresh phosphate buffer to compensate for the volume. After collecting the samples in 72 hr, their absorption was measured by spectrophotometry at 220 nm and the drug release was calculated using the following formula.

Cell culture

Two human breast adenocarcinoma cell lines, MCF7 and MDA-MB-231, were purchased from Institute Pasteur of Iran. Cultivation of cells in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 1% antibiotics (100 μg/mL streptomycin ,100 U/mL penicillin) at 37 °C, 95% humidity and 5% atmospheric CO_2 was performed.

Cell cytotoxicity assay

MTT test was used to evaluate the toxicity of mesoporous silica nanoparticles, free drugs and loaded nanoparticles (fisetin, metformin, fisetin/metformin) on MCF7 and MDA-MB-231 cell lines. First, cancer cells were cultured with a cell density of 8×10^3 cells/well in 96-well plates. After 24 hr, the cells were treated with different concentrations of MSNs (0-200 μg/mL) as well as free and drug-loaded nanoparticles (0-5 mM for metformain and nano-metformin, 0-175 μM for fisetin and nanofisetin) for 48 hr. After this time, 20 μl of MTT solution was added to each well, and after 3 hr of incubation of the cells at 37 ° C, the supernatant was removed and 100 μL of DMSO was added to each well as a solvent for the formazan crystals. After reading the absorbance at a wavelength of 490 nm, cell survival at each concentration was measured relative to the control.

Synergism analysis

To determine the effect of the simultaneous combination of metformin and fisetin, Compusyn software was used and the combination index (CI) of these compounds was calculated. CI>1 indicates antagonism effect, CI=1 indicates additivity and CI<1 indicates synergism effect. The cytotoxicity data obtained in the previous step were entered into the software and the graph and output results were evaluated.

Migration rate measurement

Scratch assay was used to check cell migration. When the cell density in the 6-well plates reached 95% confluency after cell cultivation, a scratch was created. After the scratch, the cells were treated with fisetin, metformin and a combination of both fisetin and metformin loaded in MSNs. This time is considered as zero time. The amount of cell migration is recorded by taking images at 0, 48, 24 and 72 hr. The amount of scratch area was calculated with ImageJ software.

$$\text{Release rate} = \frac{\text{Concentration taken out of dialysis bag} \times \text{Volume}}{\text{Concentration inside the dialysis bag} \times \text{The volume inside the bag}} \times 100$$

Nanomed. J. 10(4): 293-303, Autumn 2023

295

Colony formation assay

500 cells were seeded in each well of a 12-well plate to investigate the efficiency of fisetin, metformin and combined fisetin/metformin and drug-loaded MSNs in inhibiting colony formation. 24 hr later, the cells were treated with free or loaded drugs. After 48 hr, the media of the cells were removed and replaced with fresh medium. Cells were incubated at 37 °C under CO_2 atmosphere for 14 days. Afterwards, in order to observe the colonies, the cells were fixed with a solution of methanol and acetic acid with a ratio of 1:7 for 20 min. In the next step, 1 mL of crystal violet colored solution was added to each well and incubated for 2 hr at room temperature. After washing the cells with distilled water, the number of colonies were counted and compared with the control sample.

Gene expression analysis

In order to investigate the effect of the fisetin, metformin, combination of metformin/fisetin both in free form and drug-loaded MSNs on the miR-34a and miR-200b expression in cell lines, qPCR was used. In this method, MCF-7 and MDA-MB-231 cells were exposed to IC_{30} concentration of these compounds for 48 hr, then total cell RNA was extracted with TRIzol reagent (Invitrogen). For each sample, cDNA was produced using RNAs extracted with the MMLV enzyme and specific primers for miRNA synthesis (stem-loop primer). Real-time PCR was performed with SYBR®Green one-step RNA PCR kit and U6 gene was used as internal control. Interpretation of the results was done with the $2^{-\Delta\Delta Ct}$ method. The results were obtained after three independent tests conducted in the Rotor-Gene Q machine in triplicates.

Statistical analysis of data

Statistical analysis of data was done with T-test and ANOVA in SPSS software. Also, *P* value less than 0.05 is considered as a significant difference. The presented results are the result of two independent experiments in triplicates.

RESULTS
Synthesis and characterization of functional MSNs
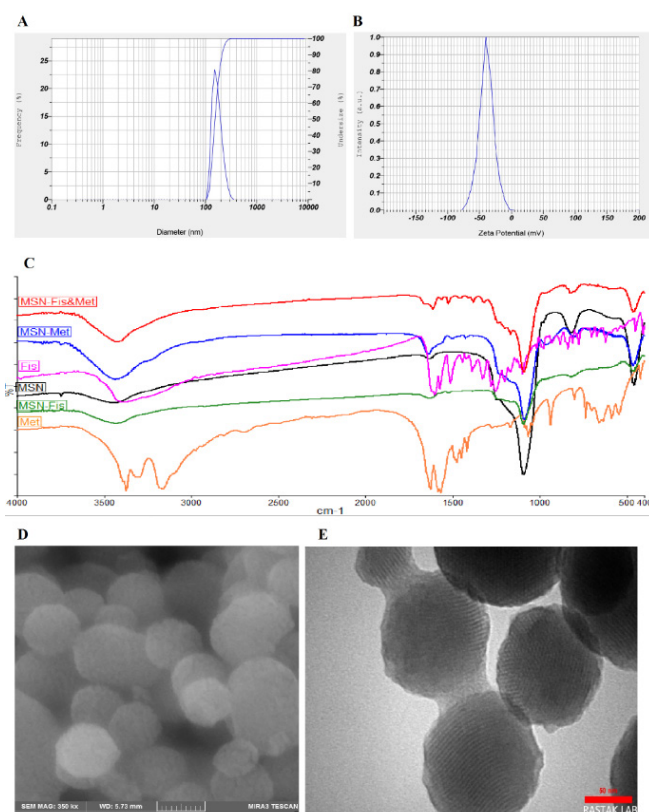
MSN had a particle size of approximately

Fig. 1. Dynamic light scattering (DLS) and zeta potential results of MSN (A, B). The FTIR spectra of MSN (black), fisetin (pink), metformin (orange), MSN-FIS (green), MSN-MET (blue) and MSN-FIS-MET (red) (C). SEM (Scale 100 nm) and TEM images (50 nm) of MSN (D &E). FIS: fisetin, MET: metformin, MSN: mesoporous silica nanoparticles

296

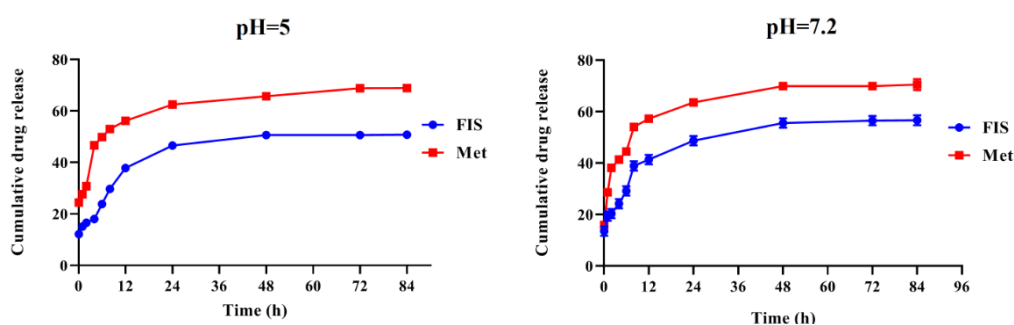*Nanomed. J. 10(4): 293-303, Autumn 2023*

Fig. 2. Fisetin and metformin release curves from fisetin/metformin co-loaded MSN. There is no significant difference in the release of metformin and fisetin from nanoparticles in acidic and neutral conditions
FIS: fisetin, MET: metformin

143.4 nm by size analyzer measurement, PDI of 0.31, and zeta-potential of -39.1 (Fig. 1A&B). Before removing the CTAB, FT-IR analysis was utilized to determine how well the template had been removed from the mesoporous materials. This was verified with bands at 2926 and 2850 cm^{-1} corresponding to the CH$_2$ and CH$_3$ groups in the structure of the CTAB molecule that were removed following template removal (Fig. 1C). SEM and TEM imaging was utilized to evaluate the morphological structure of MSNs. The TEM images revealed bright and dark areas associated with the pores and the silica walls of MSN, respectively indicating the successful synthesis of MSN (Fig. 1D&E). MSN had a particle size of approximately 100 nm in the SEM images in agreement with TEM result. Nitrogen adsorption/desorption isotherms were produced to ascertain the porosity of the MSNP and to calculate the pore volume and density. The characterizing hysteresis-type loop connected to capillary (pore) condensation at P/P0>0.2 was identified by the nitrogen absorption-desorption isotherms.

In vitro drug loading and release

For the determination of drug loading efficiency, samples underwent centrifugation and the unloaded drug present in the supernatant were measured by ultraviolet-visible (UV–vis) spectrophotometer. The absorbance of drugs in the supernatant were acquired after the centrifugation and the drug concentration was evaluated by the related standard curves. The loading efficiency of fisetin and metformin in MSN were 82% and 58%, respectively. Co-loaded efficiency of fisetin and metformin in MSN were 78% and 61%, respectively. Different pH values were used to study the drug release behavior of fisetin and metformin co-loaded in MSN (pH=5.4

and 7.2). The findings revealed that the release of metformin and fisetin from MSN was not dependent on pH (Fig. 2). The only difference was in the release amount of metformin and fisetin, and it means that in a certain period of time, the released amount of metformin was more than fisetin. The molecular weight and size of metformin is smaller than fisetin, which facilitated its release from the MSNs.

Cell viability assay

The MTT technique was used to evaluate the impact of fisetin, metformin in free and loaded in MSN on MDA-M 231 cells and MCF7 cells. Either fisetin or metformin loaded in MSN indicated more toxicity on cancer cells compared to the free forms of both metformin and fisetin. Bare nanoparticles did not show any toxicity on the cells and they were safe and biocompatible at the concentrations ranging from 10 to 5000 μM (data not shown). Cytotoxicity increased with increasing drug concentration in either free form or loaded forms. The combined treatment with metformin and fisetin showed higher toxicity on MDA-MB231 and MCF7 cells than using metformin or fisetin alone, and this toxicity increased when MSNs were used as a carrier for drug delivery (Fig. 3).

Synergism study of combination of fisetin and metformin

Synergism effect of fisetin and metformin were investigated with Compusyn software. One of the important factors in pharmacology and combination drug therapy is combination index or CI. This index shows that the combined drug has a synergism or antagonism effect.

Colony formation test

MCF7 and MDA-MB-231 cell lines were treated

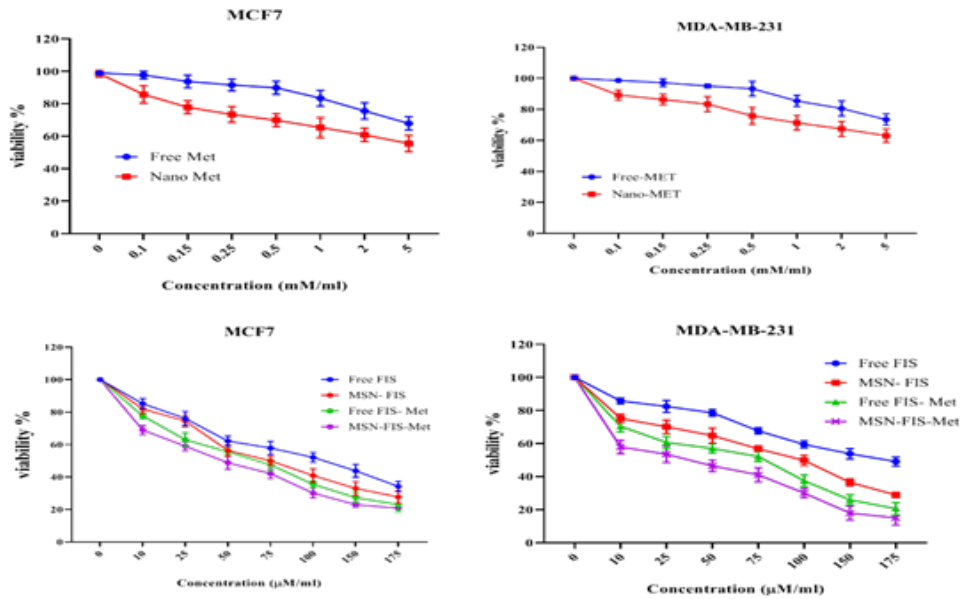*Nanomed. J. 10(4): 293-303, Autumn 2023*

297

Fig. 3. Cell viability MDA-M 231 cells and MCF7 cells after 48 hr incubation with free FIS, MSN-FIS, free MET-FIS, MSNs-MET-FIS
FIS: fisetin, MET: metformin, MSN: mesoporous silica nanoparticles

with the fisetin, metformin, metformin/fisetin combination either in free or loaded in MSNs for colony formation study. The initial number of implanted cells was 500 cells, the numbers obtained by dividing the number of colonies created by the number of implanted cells in 100. All mentioned compounds inhibited colony formation but this inhibitory effect was greater when nanoparticles were used as a carrier of medicinal compounds. The simultaneous combination delivery of fisetin and metformin in MSN (MSN-MET- FIS) showed the highest ability to inhibit colony formation in

MCF7 and MDA-MB-231 cell line (Fig. 4).

Migration assay

According to Fig. 5, with the passage of time, migration and filling of the scratch site could be observed in all samples. In the control sample, the rate of growth and filling of the scratch was higher than those treated with the fisetin, metformin, metformin/fisetin combination in free and loaded in MSNs. The amount of cell migration in free FIS, MSN- FIS, free MET/ FIS was higher than MSN-MET-FIS combined treatment. The lowest rate of cell migration was in the case of combined
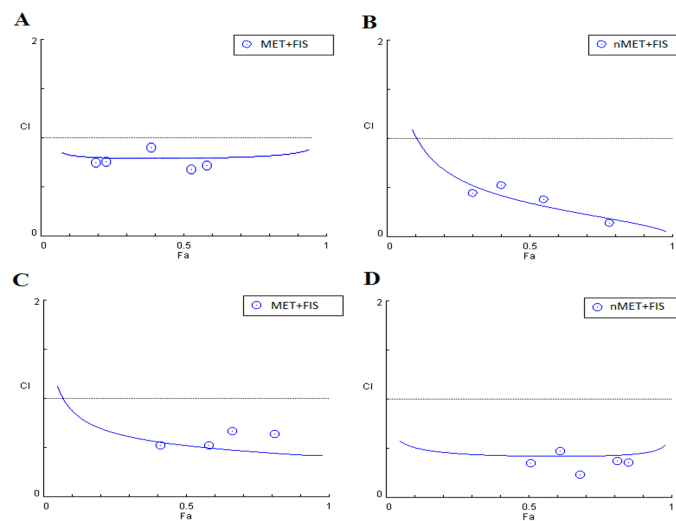

Fig. 4. Examining the synergism effect of fisetin and metformin using Compusyn software in MCF7 cells (A&B) and MDA-MB 231 cells (C&D)
FIS: fisetin, MET: metformin

298

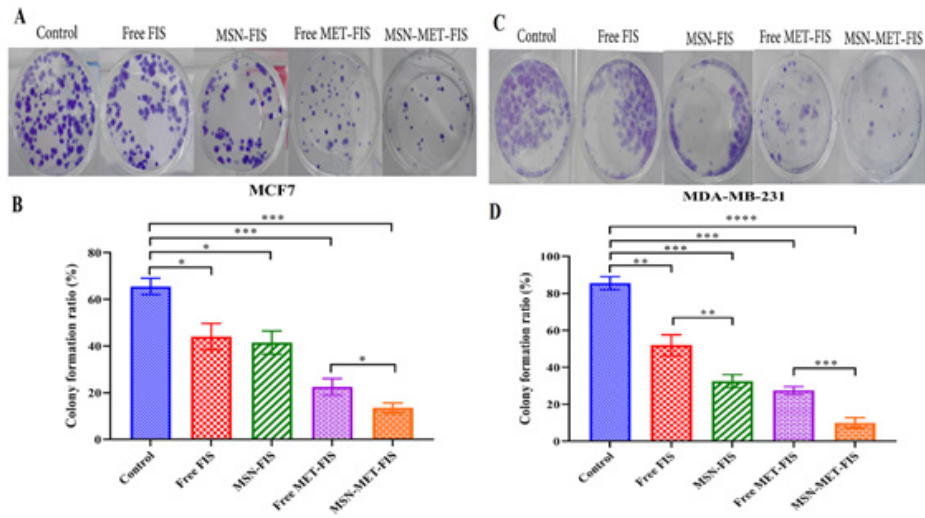*Nanomed. J. 10(4): 293-303, Autumn 2023*

Fig. 5. Investigating the effects of free FIS, MSN- FIS, free MET/ FIS, MSN-MET- FIS on colony formation in MCF7 (A&B) and MDA-MB-231 (C&D) cell line. Significant differences described by *, ** and *** between the control and treatment samples by $P<0.05$, $P<0.01$ and $P<0.001$, respectively
FIS: fisetin, MET: metformin, MSN: mesoporous silica nanoparticles

treatment of metformin and fisetin co-loaded in MSNs; Therefore, metformin and fisetin co-loaded in MSNs had a stronger migration inhibitory effect than that of the free form.

Gene expression

Treatment of cancer cells with fisetin or the combination of fisetin and metformin (free and MSN loaded) after 48 hr increased the expression of tumor suppressors miR-200b-3p and miR-34a-5p in both cell lines MCF7 and MDA-MB-231 (Fig. 6). This increase compared to the control sample was significant in all treatments, but the levels of significance were different. The increase in expression when nanoparticles were used as carriers in the transfer of fisetin or the combination of fisetin and metformin to the cells was higher compared to the free form of the
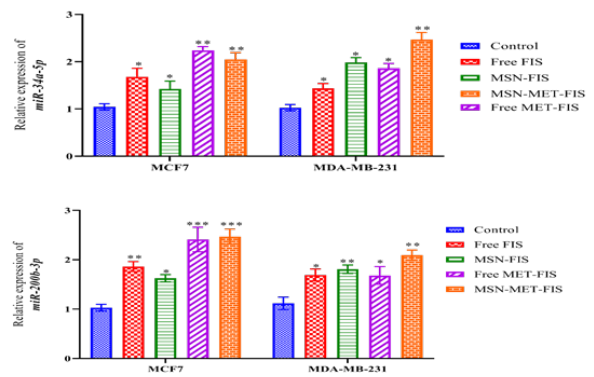

Fig. 7. The effect of free FIS, MSN-FIS, free MET/FIS, MSN-MET-FIS on miR-200b-3p and miR-34a-5p expression in MCF7 and MDA-MB-231 cell lines. Significant differences described by *, ** and *** between the control and treatment samples by $P<0.05$, $P<0.01$ and $P<0.001$, respectively
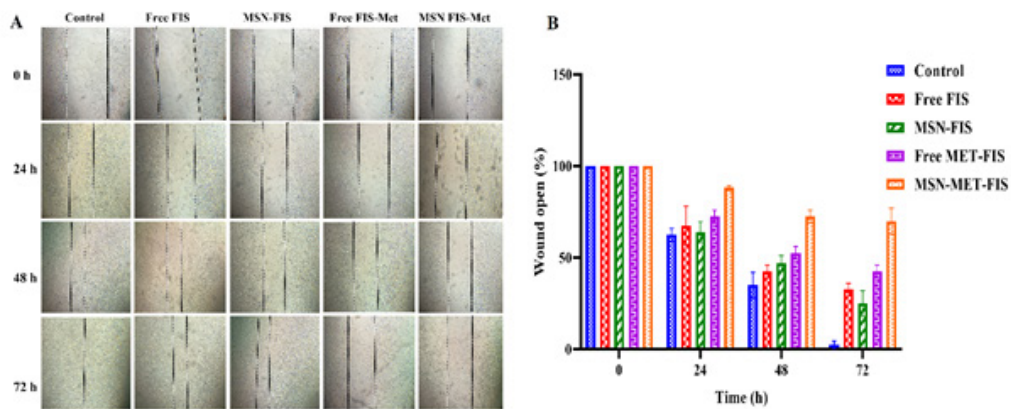FIS: fisetin, MET: metformin, MSN: mesoporous silica nanoparticles

Fig. 6. (A) MDA-MB-231 cell migration after treatment with either free or drug-loaded MSN for 72 hr. (B) The wound healing scratch ratio of control and treated cells was calculated using the ImageJ software

Nanomed. J. 10(4): 293-303, Autumn 2023

299

Table 1. miR-34a and miR-200b pathways based DIANA database

| KEGG pathway	P-value	Number of gene targets
Pathways in cancer	4.88E-08	131
Endocytosis	5.20E-08	81
Proteoglycans in cancer	5.02E-07	78
Adherent junction	1.30E-06	39
Colorectal cancer	4.56E-06	35
Cell cycle	6.80E-06	61
p53 signaling pathway	4.56E-05	39
Chronic myeloid leukemia	8.82E-05	36
Viral carcinogenesis	0.000207084	75
Thyroid hormone signaling pathway	0.000434291	51
Bacterial invasion of epithelial cells	0.000557692	36
Prostate cancer	0.000776261	41
Renal cell carcinoma	0.003172523	29
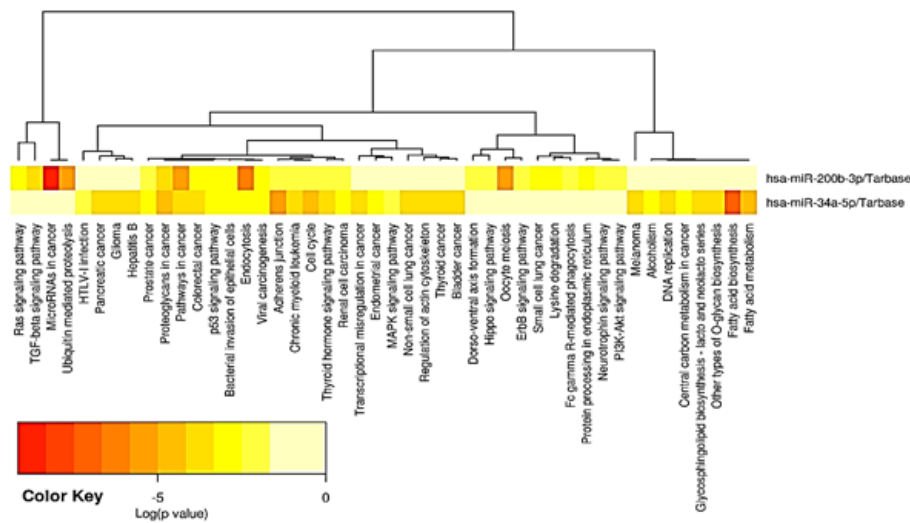
Fig. 8. heatmap of miRNAs pathways extracted from DIANA database based Tarbase miRNA targets

drugs in MDA-MB-231.

The miRNAs pathways extracted from DIANA database were displayed in Fig. 7 and top pathways were listed in Table 1. According to enrichment method, the most important pathways in which miRNAs were involved were those involved in cancer, proteoglycans in cancer, cell cycle, and p53 signaling.

DISCUSSION

Various studies have proven the antitumor effects of fisetin and metformin in different cancer cell lines. The extensive efforts to identify natural compounds derived from plants with anti-tumor properties showed that these natural compounds have little cytotoxic effects on healthy cells even at high doses. However, low solubility, poor bioavailability, and environmental and physiological instability have limited their clinical use. Nano-drug delivery formulations have become a hotspot for research because of the benefits of lower drug toxicity and enhanced drug bioavailability [32-34]. In this study, the MSN nanocarrier was used to deliver fisetin and metformin to breast cancer cells in order to improve their anticancer effects. MSN were synthesized using a base-catalyzed sol-gel method for fisetin and metformin loading, with size around 100 nm, favorable for accumulation in the tumor tissue the by EPR effect. The effect of fisetin and metformin on cell survival, colony formation and cell migration in MDA-MB-231 and

MCF7 cancer cells showed that the combined delivery of fisetin and metformin produced a greater decrease in cell survival, colony formation and cell migration compared to the treatment with each drug alone in breast cancer cells (free fisetin). This result confirms the synergistic effect of combined treatment of fisetin and metformin. The synergistic effects of metformin in combination with different medicinal agents have been proven in previous studies [35]. Researches showed that a nanoformulation of the Met–Cur combination can kill breast cancer cells faster than single-drug treatment while reducing cell cytotoxicity [36]. Moreover, metformin anti-cancer properties were enhanced when flavone was added to it [37]. Chrysin is one of the flavone that demonstrated cytotoxic effect in breast cancer cells. The combination of chrysin and metformin has been shown to have a greater inhibitory effect on the hTERT and cyclin D1 genes than either drug alone [38]. Tangeretin is the other flovone that have the ability to improve the anticancer activity of metformin. Metformin and tangeretin both had a dose-dependent inhibitory effect on the tested breast cancer cells and their doxorubicin-resistant counterparts [39]. Metformin is a drug that targets breast cancer stem cells specifically, when paired with chemotherapy, it greatly lowers breast tumor development and prolongs remission [18].

The greatest decrease in cell survival, colony formation and cell migration were observed when MSNs were used for the delivery of fisetin and metformin, simultaneously. Fisetin is a phytoestrogen, due to its structural similarity with androgen, interferes with the binding site of androgen with the ligand. Therefore, free fisetin is internalized with higher efficiency in the estrogen receptor positive MCF7 cells compared with triple negative MDA-MB-231 cells (Fig. 2). Various studies have shown that MSNs can be suitable carriers for drugs in the treatment of various diseases [40]. Saini *et al.* reported that MSN nanoparticles alone had no toxicity but MSN loaded with gemcitabine was more toxic to pancreatic cancer cell line (MIA Paca-2) than gemcitabine alone [41]. In another study, Liu *et al.* investigated the toxicity of the designed nanosystem based on MSN in SW620 human colon cancer cell line. This nanosystem alone had no toxicity on SW620. Treatment of cells with 5-fluorouracil loaded MSN had a significant effect in reducing cell viability compared to free 5-fluorouracil [3].

Gene expression analysis showed that fisetin and metformin by increasing the expression of tumor suppressors miR-200b-3p and miR-34a-5p in the treated breast cancer cells and strengthening the control mechanisms of these miRNAs, reduce cell growth, induce apoptosis and prevent cell proliferation. MiR-200b overexpression suppressed fucosyltransferase4 and decreased the clony formation, lung metastasis, migration, invasion, and tumorigenicity in breast cancer MCF-7 and MDA-MB-231 cells [42]. Additionally, miR-34a has the ability to inhibit breast cancer cell growth and migration and induce apoptotic cell death [43]. Also, it has been discovered that miR-200b through controlling ERM in MCF-7 and MDA-MB-231, participate in breast cancer cell migration and invasion [44]. miR-34a is always down-regulated in malignancies and has been reported to function as a tumor suppressor to control tumor growth [45]. Therefore, increasing the expression of tumor suppressor genes as a therapeutic strategy can be effective in finding new and pioneering treatments in the treatment of breast cancer. Examining the regulatory gene network via MiR-200b and miR-34a showed their extensive control effect on various cell processes, thus the use of these herbal compounds in miR regulation can show a unique strategy in achieving more effective treatments in cancer.

CONCLUSION

Cheap, biocompatible and efficient MSN drug delivery system was used in this study to deliver fisetin and metformin to cancer cells due to the low solubility of fisetin in aqueous systems. After the synthesis of MSN and the loading it with fisetin and metformin, a high loading efficiency was obtained. The release of fisetin and metformin loaded in MSN was investigated in acidic and neutral pH. The results confirmed the proper performance of this nanoparticle in drug release in extracellular and intracellular conditions. In this study, it was found that the combined effect of fisetin and metformin using MSNs in inducing cell death and reducing the growth of mcf-7 and MDA-MB 231 cells was far more effective than the single treatment. The results showed that treatment with each of the compounds prevented the formation of colonies, but the combined treatment of fisetin and metformin loaded into MSN has the greatest effect on MCF-7 and MDA-231 cells. The expression of miR-200b-3p and miR-34a-5p in cell

Nanomed. J. 10(4): 293-303, Autumn 2023

301

lines treated with fisetin and metformin increased significantly, considering the tumor suppressive role of these miRs, the anti-cancer effect of these compounds was confirmed, which intdoduced the combination of fisetin and metformin as an effective option in cancer treatment. The results confirmed the effect of MSN in more effective delivery of medicinal compounds to cancer cells.

ACKNOWLEDGMENTS

Ethics Approval

This article does not contain any studies on human or animals.

CONFLICTS OF INTEREST

The authors reported no potential conflicts of interest.

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302

Nanomed. J. 10(4): 293-303, Autumn 2023

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Nanomed. J. 10(4): 293-303, Autumn 2023

303