JBUON 2019; 24(4): 1555-1561
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Inhibition of endometrial carcinoma by Kaempferol is interceded through apoptosis induction, G2/M phase cell cycle arrest, suppression of cell invasion and upregulation of m-TOR/PI3K signalling pathway

Xia Lei[1]*, Jing Guo[2]*, Yuan Wang[1], Jie Cui[2], Bei Feng[2], Yani Su[2], Hong Zhao[2], Weiwei Yang[2], Yunfeng Hu[2]

[1]Department of Gynaecology and [2]Department of Oncology, Yanan University Affiliated Hospital, Yanan, Shaanxi, China.

*These two authors contributed equally to this work.

Summary

Purpose: *The main purpose of this study was to investigate the selective anticancer effects of Kaempferol against MFE-280 endometrial carcinoma cells along with evaluating its effects on apoptotic pathway, cell cycle phase distribution, cell invasion, cell migration and m-TOR/PI3K/Akt signalling pathway.*

Methods: *Cell viability of MFE-280 endometrial carcinoma cells was assessed by MTS [(3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)] assay. Apoptosis was determined by acridine orange (AO)/ ethidium bromide (EB) double staining. Cell cycle analysis was determined by flow cytometry, while Boyden chamber assay was performed to study the effects of Kaempferol on cell migration and cell invasion, respectively. The effects of Kaempferol on the protein expression of m-TOR/PI3K/Akt signalling pathway were analysed by Western blot assay.*

Results: *Kaempferol exerted considerable and selective anticancer effects on MFE-280 endometrial carcinoma cells with IC50 of 10 μM. The anticancer effects were found to be due to activation of mitochondrial-mediated apoptotic pathway and G2/M phase cell cycle arrest. Furthermore, the results also revealed that Kaempferol significantly inhibited cell migration and cell invasion trend of these cancer cells. Our results also showed that, in comparison to the untreated cells, Kaempferol-treated cells exhibited a dose-dependent downregulation of p-mTOR, p-PI3K and p-AKT proteins. However, mTOR, PI3K and Akt expression levels remained more or less unaltered.*

Conclusions: *In conclusion, the present study indicates that Kaempferol could exert anticancer effects in MFE-280 endometrial carcinoma cells selectively and that these effects were mediated via apoptosis induction, cell cycle arrest and inhibition of m-TOR/PI3K/Akt signalling pathway.*

Key words: *endometrial cancer, kaempferol, apoptosis, cell migration, cell invasion*

Introduction

Molecules from natural sources such as plants and microbes with amazing chemical structures have proved beneficial in alleviating human disorders [1]. Over the years, more and more natural products are being screened for their bioactivities [2]. Plants and microbes have been the major sources of drugs among all the natural sources [3]. Several of the drugs that are being used currently have their origin in plants, such as artemesinin which is an anti-malarial drug that has saved millions of lives [4]. Some plant-derived molecules are being used for the management of cancers [5]. Plants

Corresponding author: Yunfeng Hu, MD. Department of Oncology, Yanan University Affiliated Hospital, No. 43, North Street, Yanan, Shaanxi 716000, China.
Tel & Fax: +86 0911 2881120, Email: hyfxyd1996@163.com
Received: 22/11/2018; Accepted: 05/12/2018

produce primary and secondary metabolites out of which humans have utilised secondary metabolites as medicines since ages [6]. The plant secondary metabolites have been categorized in different groups based on their structural properties. Flavonoids constitute a vast and prevalent group of plant metabolites [7] and have been shown to exhibit a number of anticancer effects, allowing thus to be used as drugs [7]. Kaempferol is an important flavonoid which has been evaluated against different types of cancer cells [8]. For example, Kaempferol inhibits the proliferation of glioblastoma cells [9]. However, the anticancer properties of Kaempferol have not been investigated against endometrial carcinoma cells. Endometrial carcinoma is one of the devastating cancers of the female genital tract and causes considerable number of deaths throughout the world [10]. The endometrial cancer patients usually show poor prognosis in advanced stages and the currently available treatments have a number of adverse effects [11]. Herein, the anticancer effects of a plant flavonoid Kaempferol were examined against the MFE-280 endometrial carcinoma cells.

The main purpose of the current study was to investigate the anticancer effects of Kaempferol against human endometrial carcinoma along with evaluating its effects on cellular apoptosis, cell cycle arrest, cell invasion and modulation of mTOR/PI3K/AKT signalling pathway.

Methods

MTS cell viability and colony formation assay

The anticancer effects of Kaempferol were examined by MTS assay. In brief, the wells of the 96-well plate were cultured with 2×10^4 MFE-280 human endometrial cells per well, incubated overnight and then subjected to treatment with varied concentrations of Kaempferol for various time intervals. Following incubation at 37°C, MTS solution was added to the cells and absorbance was measured at 490 nm using ELISA plate reader. The effect of Kaempferol on the formation of MFE-280 colonies was investigated as described earlier [12].

AO/EB staining for apoptosis

For detection of apoptosis, the endometrial carcinoma MFE-280 cells (0.6×10^6) were grown in 6-well plates. Following an incubation period of around 12 h, the MFE-280 cells were subjected to Kaempferol treatment for 24 h at 37°C. As the cells sloughed off, 25 µl cell cultures were put onto glass slides and subjected to staining with AO and EB solution (1 µl). The slides were cover-slipped and examined with a fluorescent microscope.

Cell cycle analysis

To analyse the effect of Kaempferol on the distribution of the endometrial carcinoma MFE-280 cells in different cycle phases, flow cytometry after propidium iodide (PI) staining was performed as described previously [13]. In brief, the MFE-280 cells were grown in 6-well plates and treated with Kaempferol for 24 h. The cells were then collected and washed with phosphate buffered saline (PBS), followed by fixation in ethanol (70%). After overnight incubation at 4°C, the cells were PI-stained and examined by flow cytometry.

Cell migration and invasion assays

For cell migration and invasion analysis, the endometrial carcinoma MFE-280 cells were subjected to treatment with 0, 5, 10 and 20 µM Kaempferol. The cell migration and invasion assays were then performed as described previously [14].

Western blotting

After being lysed in RIPA lysis buffer, the MFE-280 cancer cells were subjected to bicinchoninic acid (BCA) assay for estimation of proteins. The samples were then loaded on the SDS-PAGE. The gels were then transferred to nitrocellulose membranes and subjected to treatment with primary antibodies (antibodies to p-AKT, mTOR, p-mTOR obtained from from Cell Signaling Technology, Danvers, MA, USA).] at 4°C for 24 h. After this, the membranes were incubated with HRP-conjugated secondary antibody (1:1000) for 50 min at 25°C. Enhanced chemiluminescence reagent was used to visualise the protein bands.

Statistics

SPSS software was used for statistical analyses.

Data were presented as mean±standard deviation (SD) from at least three independent experiments. Sta-

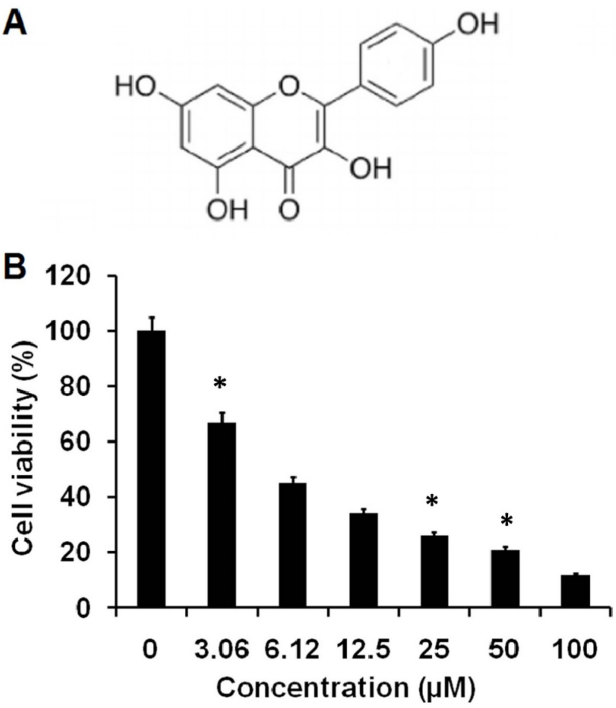

Figure 1. A: Chemical structure of Kaempferol; **B:** Effect of Kaempferol on the viability of the MFE-280 cells. The results are mean of three experiments ± SD (*p < 0.01).

tistically important difference was taken as p<0.01. One-way ANOVA with graphpad prism 7 software was used to investigate the differences among groups.

Results

Growth inhibitory effects of Kaempferol on the MFE-280 endometrial carcinoma cells

Kaempferol (Figure 1A) inhibition of the growth of MFE-280 endometrial carcinoma cells was examined by MTS assay. The results showed that that Kaempferol exerted antiproliferative effects on the MFE-280 endometrial carcinoma cell line and exhibited an IC50 of 10 μM (Figure 1B). In addition, the anticancer effects of Kaempferol on the endometrial carcinoma cells were dose-dependent. The examination of the Kaempferol treated MFE-280 cells revealed that Kaempferol could significantly inhibit the colony formation of the MFE-280 human endometrial cells (Figure 2).

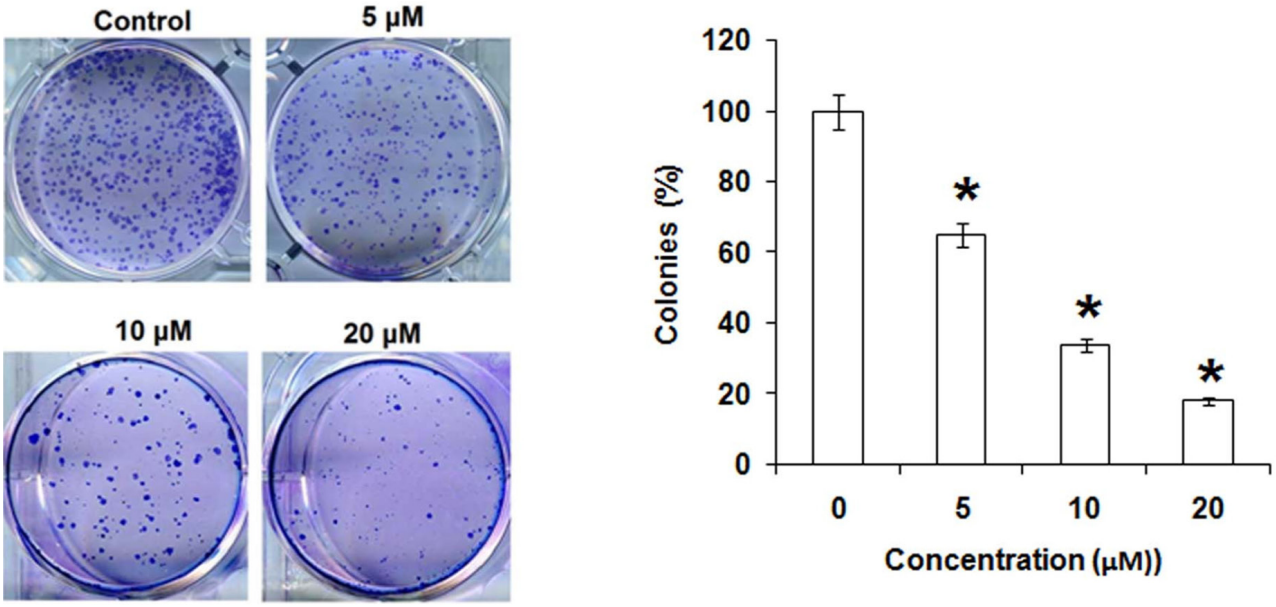

Figure 2. Inhibition of MFE-280 cell colony formation by Kaempferol. The results are mean of three experiments ± SD and reveal that Kaempferol led to colony formation cell inhibition in a dose-dependent manner (*p<0.01).

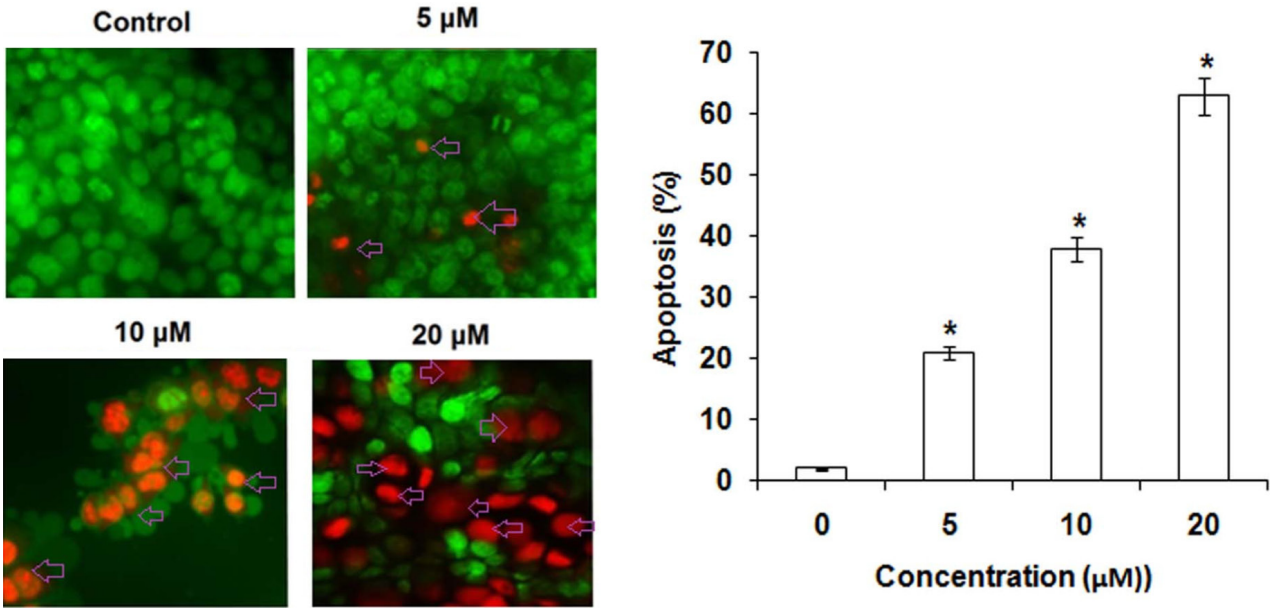

Figure 3. Effect of Kaempferol on apoptosis induction in the MFE-280 cells as depicted by AO/EB staining. The results are mean of three experiments ± SD. Red fluorescence indicates onset of apoptosis in these cells and shows that the apoptotic cells increased greatly with increasing dose (*p<0.01).

Induction of apoptosis in MFE-280 endometrial carcinoma cells by Kaempferol

The induction of apoptosis in the endometrial carcinoma MFE-280 cells by Kaempferol was checked by AO/EB staining. The outcomes of AO/EB staining showed that Kaempferol induced apoptotic cell death in the MFE-280 endometrial carcinoma cells in a dose-dependent manner (Figure 3). Analysis of the protein expression of the apoptosis biomarker proteins revealed that Kaempferol caused increase in the expression of Bax, while the expression of Bcl-2 was decreased concentration dependently (Figure 4).

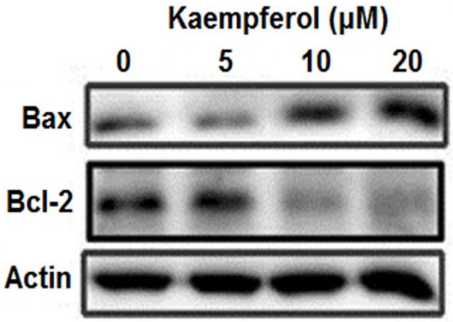

Figure 4. Effect of Kaempferol on the expression of apoptosis-related proteins in the MFE-280 cells as shown by western blotting. The experiments were performed in triplicate and show that while Bax expression increased, the expression of Bcl-2 decreased.

Kaempferol induced G2/M arrest of MFE-280 endometrial carcinoma cells

The impact of Kaempferol on the distribution of MFE-280 endometrial carcinoma cells in various cell cycle phases was assessed by flow cytometry. It was found that Kaempferol caused remarkable increase in the percentage of the MFE-280 endometrial carcinoma cells in the G2/M phase of the cell cycle. The percentage of MFE-280 human endometrial cancer cells in the G2 phase increased from 58.5 to 79.4% upon treatment with Kaempferol (Figure 5).

Kaempferol inhibited the migration and invasion of the MFE-280 cells

The effects of Kaempferol on the migration and invasion of the MF-280 cells were checked by Boyden chamber assay. It was found that Kaemp-

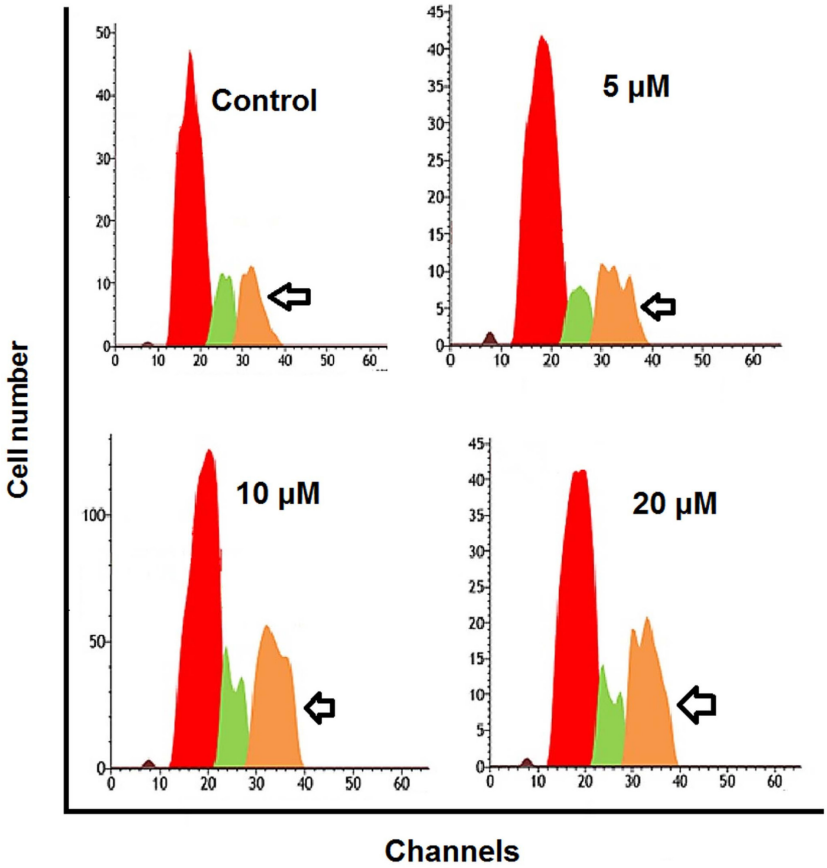

Figure 5. Kaempferol led to dose-dependent arrest of G2/M phase cell cycle distribution in MFE-280 cells. The experiments were carried in triplicate and show that the percentage of cells in G2/M phase increased significantly in a dose-dependent manner (arrows), resulting in G2/M cell cycle arrest.

ferol treatment could considerably inhibit the migration of the cancer cells in a dose-dependent fashion (Figure 6). Similar effects were also seen by Kaempferol on the invasion of the MFE-280 cells (Figure 7).

Kaempferol inhibited the PI3K/AKT/mTOR signalling pathway

The effects of Kaempferol were also investigated on the MAPK/ERK signalling pathway in MFE-280 cells. It was found that Kaempferol caused considerable decrease in the expression of p-mTOR, p-AKT and p-PI3K proteins. However, no visible effect was observed on the total mTOR, PI3K and AKT proteins expression. This effect was found to be concentration-dependent (Figure 8).

Discussion

Endometrial carcinoma is a commonly detected malignancy in women. It develops in the female genital tract and results in a considerable number of deaths world over. The used treatment methods for endometrial cancer create adverse effects on the

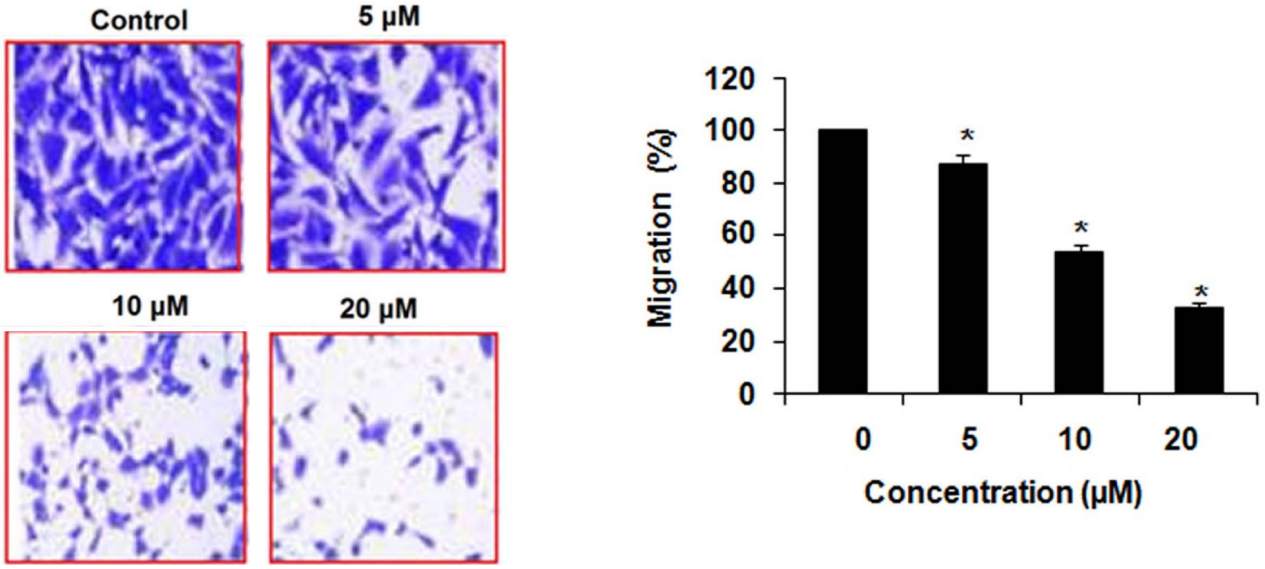

Figure 6. Effect of Kaempferol on the migration of MFE-280 cells as shown by Boyden chamber assay. The results are the mean of three experiments±SD and show an increase in the cell migration suppression with increasing dose of Kaempferol (*p<0.01).

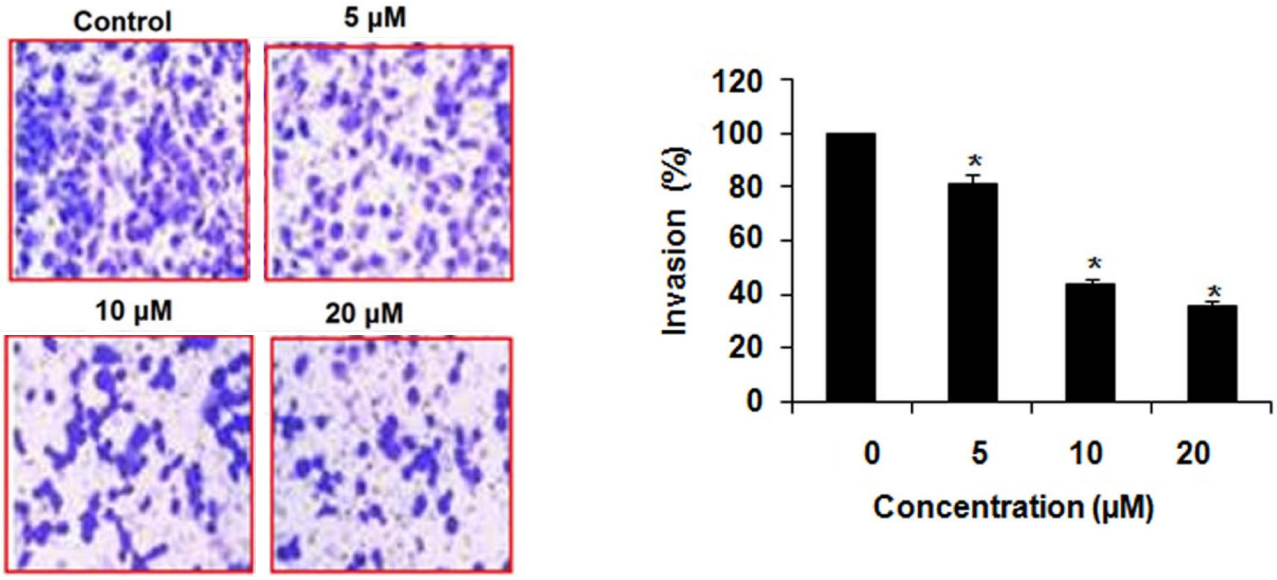

Figure 7. Effect of Kaempferol on the invasion of the MFE-280 cells as shown by Boyden chamber assay. The results are the mean of three experiments±SD and show an increase in the cell invasion suppression with increasing dose of Kaempferol (*p<0.01).

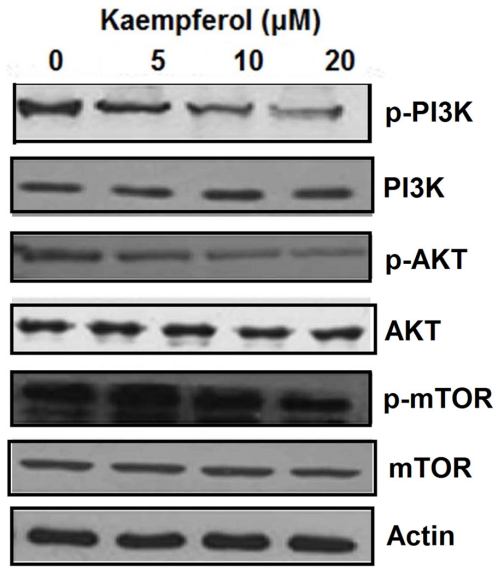

Figure 8. Effect of Kaempferol on the expression of mTOR/PI3K/AKT pathway proteins in the MFE-280 cells as shown by western blotting. The experiments were performed in triplicate. Kaempferol caused considerable decrease in the expression of phosphorylated p-mTOR, p-AKT and p-PI3K proteins. However, no visible effect was observed on the total mTOR, PI3K and AKT proteins.

health/quality of life of the patients [10]. Lack of effective drug regimes and metastasis of endometrial carcinoma limits the effectiveness of treatment of this disease [15].

Herein, the anticancer effects of the natural occurring flavonoid Kaempferol were investigated on the MFE-280 endometrial carcinoma cells. Kaempferol could inhibit the growth of the MFE-280 cells dose-dependently and exhibited an IC50 of 10 μM. Kaempferol could also suppress the colony formation of the MFE-280 endometrial carcinoma cells dose-dependently.

Previous studies have also shown that Kaempferol inhibits the growth of cancer cells. For example, Kaempferol has been found to inhibit the growth of glioblastoma and breast cancer cells [9, 16].

Next, the underlying mechanism of the anticancer activity of Kaempferol was investigated and the results showed that this molecule caused apoptosis of the MFE-280 cells. Apoptosis maintains the tissue homeostasis by eliminating the defective, abnormal or cancer cells from the body of an organism [17]. Kaempferol-triggered apoptosis was also accompanied with upregulation of Bax and downregulation of Bcl-2.

Cell cycle arrest is also responsible for the anti-proliferative activity of many anticancer drugs [13]. Herein, we found that Kaempferol blocked the division of the cells at the G2/M check point in a concentration-dependent manner. Metastasis is an important property of cancer cells [18] and the anti-metastatic potential of Kaempferol on MFE-280 cells was examined by the Boyden chamber assay and the results showed that this compound could considerably suppress the migration of cancer cells.

It has been reported that in cancer cells, a number of signal transduction pathways are overexpressed and lead to development and progression of malignancies. In this study, we investigated the effects of Kaempferol on the mTOR/PI3K/AKT signalling pathway and found that it inhibited this pathway, indicating the anticancer potential of this molecule.

Conclusion

We conclude that Kaempferol shows potent anticancer effects on the endometrial carcinoma cells by triggering the apoptotic cell death and cell cycle arrest. Besides, Kaempferol also suppresses the migration and invasion of the endometrial carcinoma cells, suggesting its potential as anticancer agent.

Acknowledgements

This study was supported by Scientific and Technological Innovation Team of Common Malignant Tumor Foundation and Clinical Research in Yanan City (No: 2015cxtd-07), and Research project on the investigation and treatment of Gynaecological Diseases in Yanan Area (No: 2016HM-04-02).

Conflict of interests

The authors declare no conflict of interests.

References

1. Kinghorn AD, Farnsworth N, Soejarto D et al. Novel strategies for the discovery of plant-derived anticancer agents. Pharmaceut Biol 2003;41(Suppl 1):53-67.
2. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016;79:629-61.

3. Patridge E, Gareiss P, Kinch MS, Hoyer D. An analysis of FDA-approved drugs: natural products and their derivatives. Drug Discov Today 2016;21:204-7.
4. Kovacs SD, van Eijk AM, Sevene E et al. The safety of artemisinin derivatives for the treatment of malaria in the 2nd or 3rd trimester of pregnancy: a systematic review and meta-analysis. PLoS One 2016 Nov 8;11(11):e0164963.
5. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 2015;14:111.
6. Rajeshkumar R, Sundararaman M. Emergence of Candida spp. and exploration of natural bioactive molecules for anticandidal therapy-status quo. Mycoses 2011;55:60-73.
7. Khan NM, Haseeb A, Ansari MY, Devarapalli P, Haynie S, Haqqi TM. Wogonin, a plant derived small molecule, exerts potent anti-inflammatory and chondroprotective effects through the activation of ROS/ERK/Nrf2 signaling pathways in human Osteoarthritis chondrocytes. Free Radical Biol Med 2017;106:288-301.
8. Chen AY, Chen YC. A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food Chem 2013;138:2099-107.
9. Nakatsuma A, Fukami T, Suzuki T, Furuishi T, Tomono K, Hidaka S. Effects of kaempferol on the mechanisms of drug resistance in the human glioblastoma cell line T98G. Die Pharmazie-An International Journal of Pharmaceutical Sciences 2010;65:379-83.
10. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. Lancet 2016;387:1094-8.
11. Burke WM, Orr J, Leitao M et al. Endometrial cancer: a review and current management strategies: part II. Gynecol Oncol 2014;134:393-402.
12. Borowicz S, Van Scoyk M, Avasarala S et al. The soft agar colony formation assay. J Vis Exp 2014;92: 51998.
13. Li JP, Yang YX, Liu QL et al. The investigational Aurora kinase A inhibitor alisertib (MLN8237) induces cell cycle G2/M arrest, apoptosis, and autophagy via p38 MAPK and Akt/mTOR signaling pathways in human breast cancer cells. Drug Design Develop Ther 2015;9:1627.
14. Kathagen-Buhmann A, Maire CL, Weller J et al. The secreted glycolytic enzyme GPI/AMF stimulates glioblastoma cell migration and invasion in an autocrine fashion but can have antiproliferative effects. Neurooncology 2018;20:1594-1605.
15. Gibson WJ, Hoivik EA, Halle MK et al. The genomic landscape and evolution of endometrial carcinoma progression and abdominopelvic metastasis. Nat Genet 2016;48:848.
16. Choi EJ, Ahn WS. Kaempferol induced apoptosis via cell cycle arrest in human breast cancer MDA-MB-453 cells. Nutr Res Practice 2008;2:322-5.
17. Mohammad RM, Muqbil I, Lowe L et al. Broad targeting of resistance to apoptosis in cancer. Semin Cancer Biol 2015;35:S78-S103.
18. Aceto N, Bardia A, Miyamoto DT et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 2014;158:1110-22.