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ORIGINAL ARTICLE

Galangin induced antitumor effects in human kidney tumor cells mediated via mitochondrial mediated apoptosis, inhibition of cell migration and invasion and targeting PI3K/AKT/mTOR signalling pathway

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Summary

Purpose: *Galangin is an important flavonoid that has been reported to be of immense pharmacological importance. It has been shown to have a number of bioactivities which range from anticancer to antimicrobial. In this study the effects of Galangin were studied on the proliferation of the A498 kidney cancer cells.*

Methods: *The antiproliferative effects were tested by MTT assay. Apoptosis was checked by subjecting the A498 cell to DAPI and annexin V/PI double staining. The effect on the cell migration and invasion was assessed by Boyden chamber assays. The expression of the apoptosis-related proteins was examined by western blot analysis.*

Results: *Galangin inhibited the proliferation of the kidney cancer A498 cells. The IC_{50} of Galangin against the A498 cancer cells was 15 μM. The anticancer effects of Galangin were due to induction of apoptosis in the A498 cancer cells as indicated by DAPI and annexin V/PI staining. Furthermore, Galangin could increase the expression of Bax, Cyt-c and decrease the expression of Bcl-2. Galangin could also inhibit the migration and invasion of the kidney cancer cells and also suppress the expression of some of the important proteins of the PI3K/AKT/mTOR signalling pathway.*

Conclusion: *In conclusion, Galangin could prove a useful new molecule in the treatment of kidney carcinoma.*

Key words: *apoptosis, cell migration, galangin, invasion, kidney cancer*

Introduction

Kidney cancer causes significant mortality and its incidence is continuously increasing [1]. It mainly arises from the renal pelvis or renal parenchyma. Even though there are some non-surgical treatment options available for kidney cancer, these are often accompanied with adverse effects and frequent relapses, making this disease even more difficult to treat [2]. It is believed that identification of efficient anticancer molecules with lower or no side effects may prove useful in the treatment of kidney cancer [3]. Natural products (especially plant-derived molecules) may prove useful in the treatment of cancer in general and kidney cancer in particular [4]. A number of molecules that exhibit anticancer activities have been isolated from plants [5]. With the huge diversity of chemical scaffolds that plants produce, there is very high possibility of isolating new molecules with better activity [6]. Among plant natural products, flavonoids form a very important group of plant secondary metabolites. Several activities have been attributed to flavonoids and since flavonoids are ubiquitously present in edible plants, they are consumed by humans in large amounts through their diet. Additionally, they are also considered non-toxic for human consumption [7]. Sev-

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eral of the plant derived flavonoids exhibit anticancer activities and many studies are being directed towards evaluating the anticancer activity of more and more flavonoids [8]. Galangin is an important flavonoid isolated from several plant species such as *Helichrysum aureonitens* [9]. Galangin has been reported to exert a number of bioactivities and has been shown to exhibit anticancer and antimicrobial activities among others [10]. However, the anticancer activity of Galangin has not been examined against the kidney cancer cells.

In this study the anticancer activity of Galangin was evaluated in the kidney cancer cell line A498. The results showed that Galangin reduces the viability of the A498 cancer cells dose-dependently and its effects were found to be due to apoptotic cell death of the A498 kidney cancer cells. Furthermore, Galangin could also inhibit the migration and invasion of the kidney cancer and also inhibited the PI3K/AKT signalling pathway. Taken together, Galangin can prove useful for the treatment of kidney cancer.

Methods

Assessment of cell viability

Galangin was procured from Sigma Aldrich, USA, and its impact on the viability of A498 cancer cells, procured from Cancer Research Institute of Beijing, China, was assessed by MTT assay. Briefly, the A498 cells were cultured at a density of 1×10^6 cells per well in 96 well plates for 12 hrs. The cells were subsequently treated with 0-100 µM Galangin for 24 hrs. Thereafter, MTT solution (20 µL) was added to each well before the addition of 500 µl of dimethyl sulfoxide (DMSO). For solubilizing the formazan formed due to MTT crystals, DMSO (500 µL) was added. Thereafter, the absorbance was measured at 570 nm by ELISA plate reader.

DAPI and annexin V/PI staining for mitochondrial apoptosis

A498 cells were cultured in 6-well plates with 1×10^6 cells per well and then treated with 0, 10, 20 and 40 µM Galangin for 24 hrs. This was immediately followed by DAPI staining. Then, the cell samples were examined and photographed with a fluorescence microscope. For estimation of apoptotic cell populations, the A498 cells were treated with Annexin V/FITC and propidium iodide (PI) for 20 min and the percentage of apoptotic cells was evaluated by flow cytometry (BD Biosciences, San Jose, CA, USA).

Assessment of cell migration and invasion

The effect of Galangin on the migration of A498 cells was examined by the Boyden chamber assay. Two percent of fetal bovine serum (FBS) was used to suspend the A498 cells, which were then placed in upper chamber with transwells having 8 µm pore size. Afterwards, Dulbecco's modified Eagle medium (Invitrogen Life Technologies, MA, USA) supplemented with 10% FBS was added to the lower chamber. These cells were then incubated for 24 hrs at 37°C. The non-migrated A498 cells were removed from the upper side of the membrane while the migrated cells on the lower surface were subjected to fixation with 100% ethanol, followed by Giemsa staining. Cell migration was determined by estimating the number of the migrated cells by microscopy. The cell invasion assay was performed by transwell assay similarly to the migration assay described above, except that the pores were coated with Matrigel.

Assessment of protein expression by western blotting

The A498 cells were lysed by lysis buffer and the concentration of the protein in each cell lysate was determined by bicinchoninic acid assay (BCA). The expression of the proteins of interest was determined by western blotting as described previously [11].

Statistics

The experiments were performed in triplicate and the data are presented as the mean of three experiments ± standard error of the mean (SEM). The data were statistically analyzed using Student's Newman Keul's test or *t*-test. $P < 0.01$ was considered to be statistically significant.

Results

Antiproliferative effects of Galangin on A498 cell line

The growth inhibitory effects of Galangin (Figure 1) were confirmed on A498 kidney cancer cell line. The A498 cells were subjected to treatment with Galangin at varied doses and it was observed that Galangin displayed significant anticancer effects against A498 cells with an observed IC_{50} of 15 µM. The effect of Galangin on the growth of A498 cells was concentration-dependent (Figure 2).

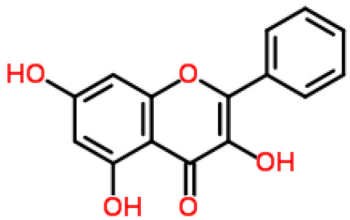

Figure 1. Chemical structure of Galangin.

Induction of apoptosis in A498 cells

To know about the reason behind the anticancer effects, Galangin-treated A498 cells were subjected to DAPI staining. It was observed that Galangin caused apoptosis in A498 cells dose-dependently (Figure 3). The analysis of apoptotic cell population was performed flow-cytometrically after annexin V/PI staining (Figure 4). The apop-

totic A498 cells increased from 6.2 % in the control to 50.1 % at 30 μM dose of Galangin. To assess if apoptosis was due to the activation of mitochondrial apoptotic pathway, the protein expression of Bax, Bcl-2 and Cyt-c was examined. The results revealed that the protein expression of Bax and Cyt-c was increased in a dose-dependent manner and that of Bcl-2 was decreased (Figure 5).

Galangin inhibited cell migration and invasion

The effect of Galangin on cell migration was also assessed on A498 cells (Figure 6). The results of cell migration assay after Galangin treatment at 15 μM concentration for 24 hrs indicated that Galangin reduced the motility and migration of the A498 cells in a dose-dependent manner. Additionally, Galangin also inhibited significantly the A498 cell invasion (Figure 7).

Galangin suppressed the PI3K/AKT/mTOR signalling pathway

Finally, as part of our ongoing research, the effect of Galangin on the PI3K/AKT/mTOR cascade was evaluated. The results revealed that although there was no effect on the expression of PI3K, AKT and mTOR proteins, the expression of p-PI3K, p-AKT and p-mTOR proteins was significantly decreased (Figure 8).

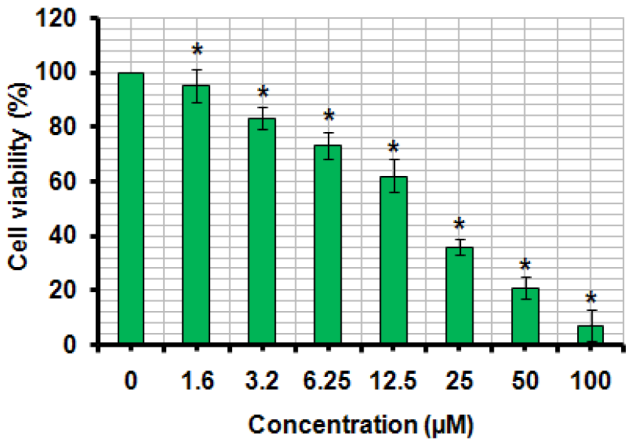

Figure 2. Effect of Galangin on the viability of A498 cells by MTT assay. The experiments were repeated three times and shown as mean ± SD (*p <0.01). The Figure clearly shows that Galangin inhibits A498 cells viability in a concentration-dependent manner.

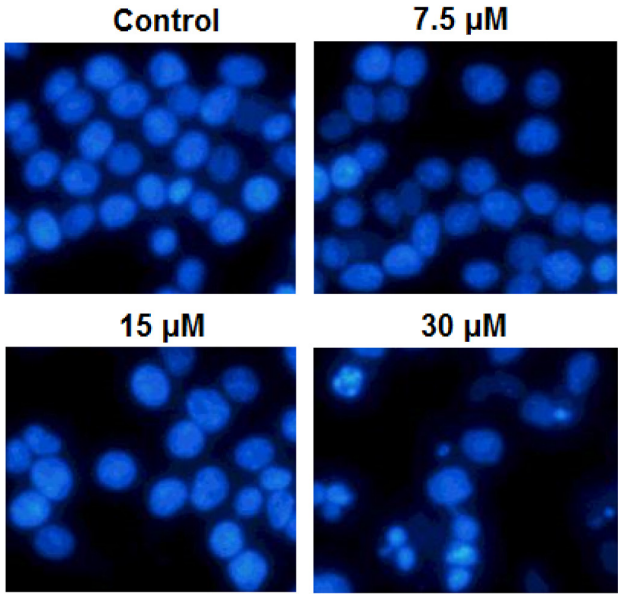

Figure 3. Galangin induced apoptosis in A498 cells as indicated by DAPI staining. The experiments were repeated three times. The Figure shows that Galangin induced apoptosis in a concentration-dependent manner.

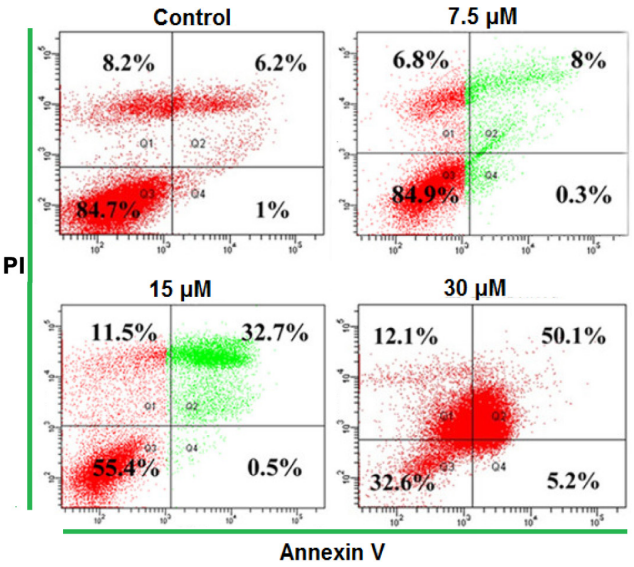

Figure 4. Estimation of the percentage of the apoptotic A498 cells. The experiments were repeated three times. The Figure depicts that the percentage of apoptotic cells increased with the increase in the concentration of Galangin.

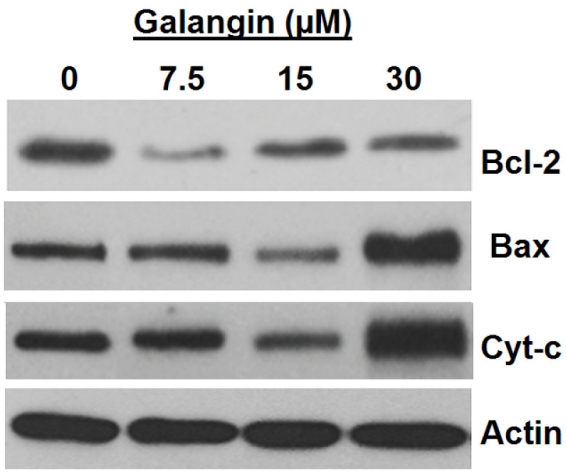

Figure 5. Effect of Galangin on Bax, Bcl-2 and Cyt-c expression as indicated by western blotting. The experiments were repeated three times. The Figure reveals that the expression of Bcl-2 is decreased and of Bax and Cyt-c is increased upon treatment with Galangin.

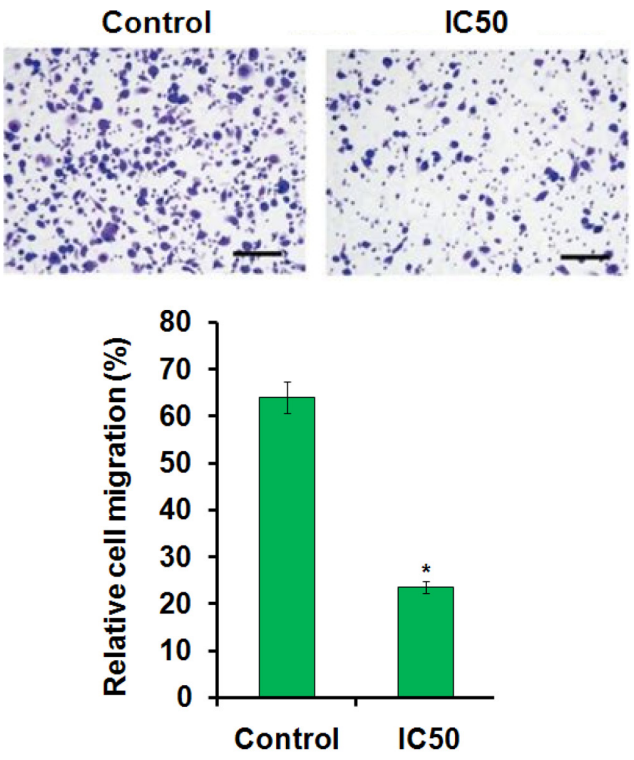

Figure 6. Effect of Galangin on the migration of A498 cells at IC_{50} showing strong migration inhibition. The experiments were repeated three times and shown as mean ± SD (*p <0.01).

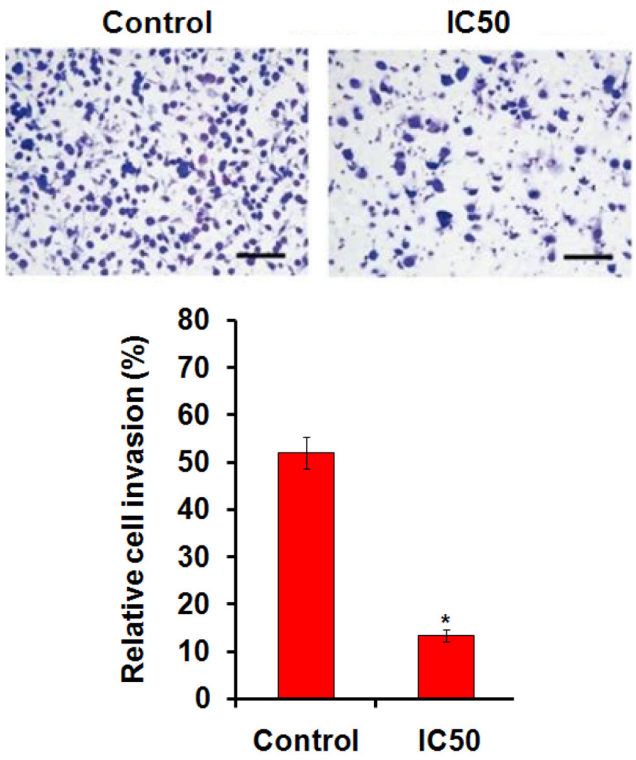

Figure 7. Effect of Galangin on the invasion of A498 cells at IC_{50} showing strong invasion inhibition. The experiments were repeated three times and shown as mean ± SD (*p <0.01).

Discussion

Kidney cancer is one among the deadly malignancies and the treatment strategies for it are limited, do not give satisfactory results and have several side effects. Therefore, there is an imperative need to identify less toxic and more effective plant-derived compounds for the management of this disease [12]. In the present study we screened Galangin against the A498 kidney cancer cells and attempted to explore the underlying mechanisms of its anticancer activity. The results showed that Galangin decreases the viability of A498 kidney cancer cells. These antiproliferative effects exhibited a concentration-dependent trend as the cell viability decreased with increase in the concentration of the A498 kidney cancer cells. These observations are in agreement with previous investigations wherein Galangin has been found to inhibit the proliferation of cancer cells. For example, Galangin has been reported to inhibit the proliferation of ascites carcinoma in rats [13]. Similarly, Galangin has been observed to inhibit the growth of colorectal adenocarcinoma cells [14]. Next, to investigate the underlying mechanisms of Galangin on 498 cells we used DAPI and annexin V/PI staining and the results showed that Galangin prompted apoptosis in A498 kidney cancer cells. The apoptosis

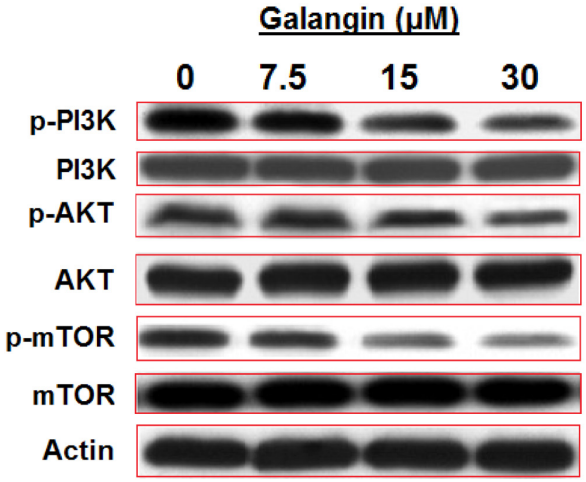

Figure 8. Effect of Galangin on the PI3K/AKT/mTOR pathway (western blot) and depicts that Galangin inhibits the expression of the PI3K/AKT signaling pathway proteins in a concentration-dependent manner. The experiments were repeated three times.

inducing property of Galangin should be considered important as it has been reported that induction of apoptosis in cancer cells prevents the development of chemoresistance [15]. Furthermore, Galangin has been reported to induce apoptosis in hepatocellular carcinoma via the mitochondrial pathway [16]. To confirm whether Galangin induc-

es apoptosis via mitochondrial/intrinsic pathway we checked the expression of Bax, Bcl-2 and cyt-c. The results showed that Galangin increased the expression of Bax and cyt-c while the expression of Bcl-2 was found to decrease in a concentration-dependent manner. In yet another study, it was reported that Galangin inhibits the migration of the renal carcinoma cells [17] and therefore we examined the impact of Galangin on the migration and invasion of A498 kidney cancer cells. What we found was that Galangin inhibits the migration and invasion ofA498 kidney cancer cells as well. PI3K/AKT/mTOR signalling pathway is one of the important pathways which has been reported to be activated in cancer cells [18] and in this study it was observed that Galangin could suppress the expression of some of the important components of this pathway. Taken together we conclude that Galangin could prove to be a lead molecule in the treatment and management of kidney cancer.

Conclusion

Galangin inhibits the proliferation of the kidney cancer cells. Its antiproliferative activity was due to induction of mitochondrial apoptosis. Besides, Galangin could also inhibit the cell migration, invasion as well as the important PI3K/AKT/mTOR signalling pathway. Therefore, Galangin could prove to be an important molecule for the treatment of kidney cancer and should be further evaluated *in vivo*.

Conflict of interests

The authors declare no conflict of interests.

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