Journal of Animal and Veterinary Advances 12 (3): 352-359, 2013
ISSN: 1680-5593

Mangiferin Induces Apoptosis and Cell Cycle Arrest in MCF-7 Cells Both *in vitro* and *in vivo*

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Abstract: Mangiferin, a C-glucosylxanthone (1, 3, 6, 7-tetrahydroxyxanthone-C2-β-D-glucoside) purified from plant sources was shown to have *in vitro* growth-inhibitory and apoptosis-inducing activity against MCF-7 cells and it also possessed anti-tumor property on MCF-7 xenograft mice *in vivo*. Mangiferin triggered G_2/M phase cell-cycle arrest via down-regulating cdc2-cyclinB1 singling pathway and induced apoptotic cell death through inhibiting PKC-NFκB pathway in human breast carcinoma MCF-7 cells. In addition, mangiferin had anti-cancer effects *in vivo* and it could decrease the volume and weight of subcutaneous tumor mass obviously as well as expanded lifespan of xenograft mice. With the molecular mechanisms of mangiferin-induced anti-tumor activities were gradually clarified, traditional Chinese medicine would become potential anti-neoplastic drugs in future cancer therapeutics.

Key words: Mangiferin, cdc2-cyclinB1 singling pathway, PKC-NFκB pathway, drugs, tumor

INTRODUCTION

Mangiferin is isolated from the leaves, stem bark, fruit peels and root of *Mangiferina indica* L. as well as many other herbal species (Sanchez *et al*., 2000). Mangiferin possesses many beneficial biological activities including antioxidant (Dar *et al*., 2005), antitumor (Guha *et al*., 1996), anti-bacteria, antiviral (Guha *et al*., 1996) and immunomodulatory activities. Mangiferin has remarkably anti-neoplastic effects toward breast cancer (Garcia-Rivera *et al*., 2011; Noratto *et al*., 2010), colon cancer (Noratto *et al*., 2010; Chieli *et al*., 2009), leukemia (Garcia-Rivera *et al*., 2011; Cheng *et al*., 2007; Peng *et al*., 2004; Percival *et al*., 2006), lung cancer (Garcia-Rivera *et al*., 2011; Chari *et al*., 2009) and prostate cancer (Garcia-Rivera *et al*., 2011). Earlier reports have shown that mangiferin remarkably inhibited K562 leukemia cell proliferation and induced cell apoptosis through down-egulation of NF-κB activity (Peng *et al*., 2004). Another research demonstrated that mangiferin inhibited telomerase activity of K562 cells in a time and dose-dependent manner and that it could induce apoptosis and up-regulate the mRNA and protein levels of Fas (Percival *et al*., 2006).

Cancer is a complex genetic disease resulting from mutations of oncogenes or tumor suppressor genes that would lead to the alteration of signaling pathways (Chari *et al*., 2009). It is widely acknowledged that normal cells are able to check and repair DNA damage when cells are continuously subjected to external stimuli, otherwise affected cells would commit cell death like apoptosis and autophagy if the DNA lesion cannot be repaired (Hanahan and Weinberg, 2000). Dysfunction of repair or elimination of damaged cells leads to malignant transformation eventually and thus PCD modulation could function as a potential target of cancer treatment by which damaged and potentially deleterious cells could be cleared (Chari *et al*., 2009). Apoptotic cells have long been observed to display a series of morphological characteristics, e.g., shrinkage of cytoplasm and nucleus, membrane blebbing and shattering (Hartwell and Kastan, 1994; Vermeulen *et al*., 2003; Malumbres and Barbacid, 2009) suggesting the existence of common pathways involved in these apoptotic cell death which later proved to be the caspase family. Usually but not exclusively, it associates with the activation of caspase

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and both of the extrinsic and intrinsic apoptotic pathways finally converge to a common process that is initiating caspase cascade.

Herein, researchers showed that mangiferin could trigger G_2/M phase cell-cycle arrest via downregulating cdc2-cyclinB1 singling pathway and could induce apoptosis by inhibiting PKC-NFκB pathway in human breast carcinoma MCF-7 cells. In addition, mangiferin also had anti-cancer effects *in vivo* and it could decrease the volume and weight of subcutaneous tumor mass obviously as well as it could expand lifespan of MCF-7 xenotransplant mice.

MATERIALS AND METHODS

Reagents: Mangiferin was purified and maintained by the lab and human breast adenocarcinoma MCF-7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Fetal Bovine Serum (FBS) was purchased from Gibco BRL (Grand Island, NY, USA). 3-(4,5-dimetrylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), z-VAD-fmk (pan-caspase inhibitor), z-DEVD-fmk (caspase-3 inhibitor), z-IETD-fmk (caspase-8 inhibitor) and z-LEHD-fmk (caspase-9 inhibitor) were purchased from Sigma Chemical (St. Louis, MO, USA).

Cell culture: Human breast adenocarcinoma MCF-7 cells were cultured in DMEM medium containing 10% FBS, 100 mg mL^{-1} streptomycin, 100 U mL^{-1} penicillin and 0.03% L-glutamine and maintained at 37°C with 5% CO_2 at a humid atmosphere. In order to form an *in vivo* cancer model, the cultured human breast adenocarcinoma MCF-7 cell suspension (1.0×10^7 cells) was inoculated on the neck of 3 months old male C57BL/6J mice.

The MTT colormetric assay: MCF-7 cells at logarithmic growth phase were seeded in a 96 well plate and incubated at 37°C for 24 h and various concentrations of mangiferin (12.5, 25, 50 and 100 μg mL^{-1}) were added for incubating with 12, 24, 36 and 48 h, respectively while control group was treated with PBS. The 0.05 mg (10 μL of 5 mg mL^{-1}) MTT was added to each well and incubated at 37°C for 4 h and then medium was removed followed by shaking thoroughly for 1 h. Finally, termination buffer was added into each well. The absorbance at 570 nm was measured with a spectrophotometer [Model 3550 Microplate Reader (Bio-Rad)]:

$$\text{Cell viability (\%)} = \left(\frac{\text{OD 570nm (drug)}}{\text{OD 570 nm (control)}}\right)\times 100$$

Observations of cell morphologic changes: MCF-7 cells were seeded into 6-well culture plate at a density of 4×10^5 cells/well in DMEM (GIBCO) containing 10% FBS and cultured for 24 h. Control groups were treated with 0.05% DMSO while mangiferin groups were treated with 25 μg mL^{-1} mangiferin. Hoechst 33258 staining was applied to further detect apoptotic nuclear morphology changes. Cells were fixed with 4% paraformaldehyde for 30 min at room temperature after 24 h incubation with or without mangiferin and then cells were washed twice with PBS. hit24Hoechst 33258 (5 μg mL^{-1}) was added and stained for 15 min and cells were washed and analyzed immediately with fluorescence microscopy. (Olympus, Tokyo, Japan).

Measurement of cell cycle and sub-G_1 cells: MCF-7 cells treated with mangiferin or DMSO at 37 centigrade for 12, 24, 36 and 48 h were harvested and washed with 0.01 M cold PBS and then fixed with 70% ethanol maintained at 4°C for 24 h. Cell pellets were stained with 1 mL PI solution including 65 μg mL^{-1} PI and 50 μg mL^{-1} RNase in 0.01 M PBS for 30 min at 37°C. The percentages of the cells at different phases of cell cycle or undergoing apoptosis were evaluated by Calibur FACScan flowcytometry (Becton Dickinson, Franklin Lakes, NJ).

Caspase assay: The MCF-7 cells were seeded into 6 well culture plate at a density of 4×10^5 cells/well for 14 h incubation. Subsequently, cells were treated with or without 200 μM z-VAD-fmk (pan-caspase inhibitor), z-DEVD-fmk (caspase-3 inhibitor), z-IETD-fmk (caspase-8 inhibitor) and z-LEHD-fmk (caspase-9 inhibitor) for 2 h incubation at 37°C. Mangiferin were treated for 24 h incubation and MTT assay was performed as described above. In addition, caspase-3, -8 and -9 activities were further measured by a colorimetric assay kit (Biovision) according to the manufacturer's instructions.

Detection of mitochondrial membrane potential: Mitochondrial membrane potential was measured by fluorescent dye rhodamine-123. After treatment with 25 μg mL^{-1} mangiferin for 12, 24, 36 and 48 h. Cells were collected and suspended in 1 mL of PBS containing 1 μg mL^{-1} rhodamine-123 and incubated at 37°C for 15 min. The fluorescence intensity of the cells was analyzed by FACScan flow cytometry (Becton Dickinson, Franklin Lakes, NJ).

Western blot analysis: MCF-7 cells were treated with 25 μg mL^{-1} mangiferin for 12, 24, 36 and 48 h, respectively and then both adherent and floating cells were collected.

The cell pellets were resuspended with lysis buffer and lysed at 4°C for 1 h and the lysis buffer consists of Hepes 50 mmol L^{-1} PH 7.4, Triton X-100 1%, sodium orthovanada 2 mmol L^{-1}, sodium fluoride 100 mmol L^{-1}, edetic acid 1 mmol L^{-1}, PMSF 1 mmol L^{-1}, aprotinin (Sigma, MO, USA) 10 mg L^{-1} and leupeptin (Sigma) 10 mg L^{-1}. After 12,000 g centrifugation for 15 min, the protein content of supernatant was determined by Bio-Rad Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of the total protein were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes, the membranes were soaked in blocking buffer (5% skimmed milk). Proteins were detected using primary and secondary antibodies and visualized with ECL (Amersham).

***In vivo* study design:** Fifty, 3 months old male C57BL/6 mice are randomly divided into five groups:

- In blank control group, mice administered saline after MCF-7 cells injection
- In high dose mangiferin group, mice administered 100 mg kg^{-1} mangiferin after MCF-7 cells injection
- In medium dose mangiferin group, mice administered 50 mg kg^{-1} mangiferin after MCF-7 cells injection
- In low dose mangiferin group, mice administered 10 mg kg^{-1} mangiferin after MCF-7 cells injection
- In positive control group, mice administered cisplatin (10 mg kg^{-1}) after MCF-7 cells injection

About 100, 50 and 10 mg kg^{-1} of mangiferin were injected from intraperitoneal to mice and the therapy lasted for 2 weeks. Animal handling was in accordance with Ethics Committee of Sichuan University and all animals were kept in 12 h light/dark cycle with free access to water and food which is in consistent with IVC requirement in Sichuan University.

Relative tumor volume, survival rate, inhibitory rate and body weight determination: Tumor volume was determined from caliper measurements according to the equation:

$$Tvol = Length \times Width \times Depth \times 0.5$$

And the Relative Tumor Volume (RTV) was calculated as the relative increase or decrease in mean tumor volume from the start of treatment (V_0) until the value at a given time (V_t) and RTV = V_t/V_0. Statistical analysis of data was performed by ANOVA by the Bonferroni method:

$$\text{Inhibitory rate of tumor volume} = \frac{V_{control} - V_t}{V_{control}} \times 100\%$$

After 14 days treatment, mice were killed by cervical dislocation and the subcutaneous tumor mass were determined:

$$\text{Inhibitory rate of tumor weight} = \frac{W_{control} - W_t}{W_{control}} \times 100\% \ cm^3$$

Statistical analysis of the data: All the results presented here were confirmed in at least three independent experiments. These data were expressed as mean±SEM. Statistical comparisons were made by Student's t-test and two-way ANOVA. The $p<0.05$ was considered significant difference.

RESULTS AND DISCUSSION

Cytotoxic effects of mangiferin on MCF-7 cells: To detect the anti-tumor property of mangiferin in MCF-7 cells, MTT assay was performed and the result demonstrated that mangiferin induced MCF-7 cell death in a dose-dependent manner. Mangiferin whose concentration varied from 0-100 μg mL^{-1} demonstrated that even very low dose of treatment (12.5 μg mL^{-1}) possessed inhibitory effect on MCF-7 cell proliferation (Fig. 1a). After 24 h incubation with 25 μg mL^{-1} mangiferin, the inhibitory rate reached nearly 50% and for higher dose of mangiferin (100 μg mL^{-1}), the inhibitory rate could reach >50% within 12 h. So, the inhibitory efficiency of mangiferin is comparatively high.

Observation of cell cycle distribution and cellular morphology: Subsequently, cell cycle analysis was performed to observe the cell cycle distribution of MCF-7 cells after the treatment of mangiferin. By statistics of cell numbers of the sub-G_1 phase, the G_0/G_1 phase, the S phase and the G_2/M phase, more cells were stalled in the sub-G_1 phase after addition of mangiferin. The higher dose of mangferin which was administrated, the more MCF-7 cells which were stucked in the sub-G_1 fraction which means that mangiferin induced apoptosis of MCF-7 cells in a time-dependent manner. Meanwhile, the G_2/M phase arrest was also apparent in the mangiferin treated cells (Fig. 1b). Moreover, marked apoptotic morphological changes of MCF-7 cells (treated with mangiferin) were confirmed by Hoechst 33258 staining. Nuclei in which DNA resides were round and homogeneously stained in the control group whereas the mangiferin-treated cells showed manifest fragmented DNA in nuclei (Fig. 1c). Altogether, these results indicate that mangiferin induces apoptotic cell death in MCF-7 cells.

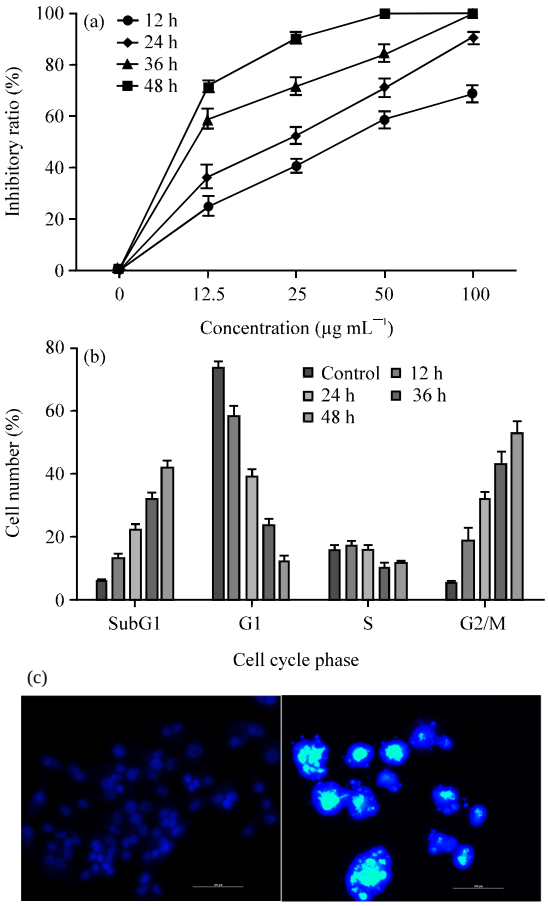

Fig. 1: The apoptotic effect of mangiferin on MCF-7 cells; a) Inhibitory ratio of MCF-7 cells with different concentration of mangiferin was determined by MTT colormetric assay; b) Cells treated with carious dosage of mangiferin were stained with PI at 37°C for 30 min and measured by flowcytometry after collection. The percentage of cells in different phases of the cell cycle was represented by a bar diagram; c) The morphologic alterations were observed with or without mangiferin under the fluorescent microscopy (200x)

Mangiferin induces apoptosis in a caspase-dependent manner: To evaluate whether caspase pathway is involved in mangiferin-induced cell death, caspase inhibitors were applied as mentioned above. After 24 h incubation with mangiferin, mangiferin-induced cell growth inhibition was completely suppressed by adding pancaspase, caspase-3, caspase-8 and caspase-9 inhibitors (Fig. 2a). It is indicated that mangiferin induces cell apoptosis in a caspase-dependent manner. Moreover, Western blot analysis of caspase-3, caspase-8 and caspase-9 activities showed that the expressions of

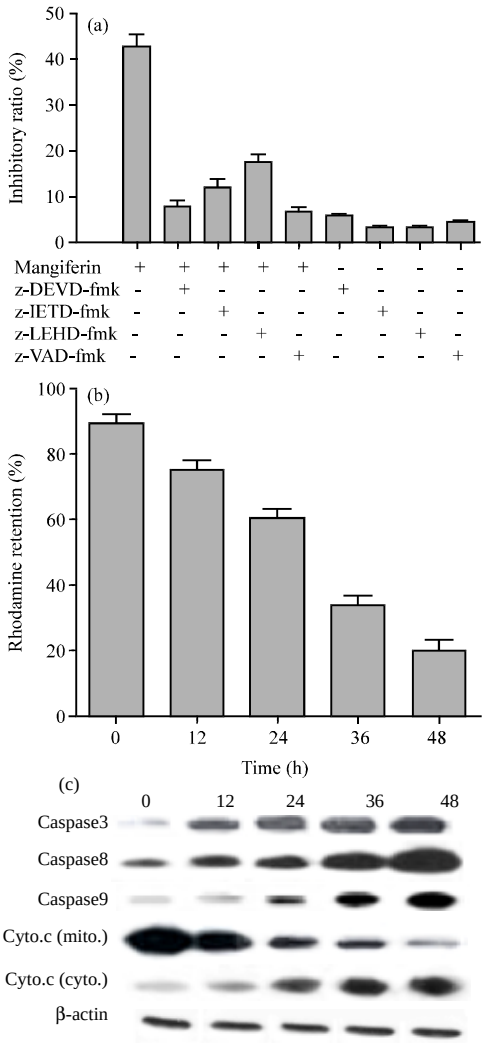

Fig. 2: Mangiferin induces apooptosis through a caspase-mediated mitochondrial pathway; a) After pretreatment with or without 200 μM z-VAD-fmk (pan-caspase inhibitor), z-DEVD-fmk (caspase-3 inhibitor), z-IETD-fmk (caspase-8 inhibitor) and z-LEHD-fmk (caspase-9 inhibitor) 2 h, the growth inhibition of MCF-7 cells treated with mangiferin for 24 h was determined (mean±SEM, n = 3); b) After treated with 27 μg mL^{-1} mangiferin for various time periods, the cells were loaded with 1 μg mL^{-1} rhodamine-123 at 37°C for 15 min and analyzed by flow cytometry and fluorescence intensity was represented by a bar diagram; c) Mangiferin-induced caspase-3, caspase-8 and caspase-9 expressing as well as cytochrome c release. The cells were treated with mangiferin for the indicated time periods followed by Western blot analysis for detecting caspase-3, caspase-8, caspase-9 and cytochrome c expressions and β-Actin was used as an equal loading control

procaspase-3, caspase-8 and caspase-9 were decreased while the expressions of caspase-3, caspase-8 and caspase-9 were increased after treated with mangiferin for 12, 24, 36 and 48 h (Fig. 2c). Therefore, these results confirmed that mangiferin increased the activities of caspase-3, caspase-8 and caspase-9 in a time-dependent manner.

Mangiferin induces apoptosis through mitochondrial pathway: To further explore whether mangiferin induced apoptosis is through mitochondrial pathway, the integrity of mitochondrial membranes was measured by rhodamine 123 staining. Staining result indicated that mangiferin decreased the fluorescence intensity of rhodamine 123 in a time-dependent manner (Fig. 2b). In addition, the amount of cytochrome c in the mitochondria of the cells was decreased as well. At the meantime, the amount of cytochrome c in the cytosol of the cells was increased which indicated that cytochrome c was released from mitochondria (Fig. 2c). These results clearly demonstrate that mangiferin-induced apoptosis in MCF-7 cells is mediated by a mitochondrial pathway.

Mangiferin induces apoptosis in MCF-7 cells through inhibiting PKC-NFκB pathway: Furthermore, MCF-7 cells were pretreated with PKC inhibitor staurospotine, NF-κB inhibitor PDTC and mangiferin-induced cell cytotoxicity was measured. As shown in Fig. 3a, these inhibitors significantly increased mangiferin-induced cytotoxicity indicating that they play a protective role in mangiferin-induced MCF-7 cell apoptosis. Western blot data showed that the treatment of MCF-7 cells with mangiferin resulted in down-regulation in PKC and NF-κB proteins levels (Fig. 3b).

Mangiferin induces G_2/M phase cell-cycle arrest through downregulating cdc2-cyclinB1 singling pathway: To examine whether mangiferin induced cell cycle arrest is caused by the downregulation of cdc2-cyclin B1 signaling pathway, MCF-7 cells were treated with 25 μg mL^{-1} mangiferin for different time periods and the cell cycle distribution was subsequently analyzed by flowcytometry. As shown in Fig. 1b, compared with that of the control group, the treatment of MCF-7 cells with mangiferin resulted in an increase in percentage of G_2/M phase cells. This result indicated that mangiferin also induced G_2/M phase arrest at early time. Subsequently, the effects of mangiferin on cell cycle-regulatory molecules including cyclinB1 and cdc2 were determined by Western blotting. As shown in Fig. 3b, the treatment of MCF-7 cells with mangiferin

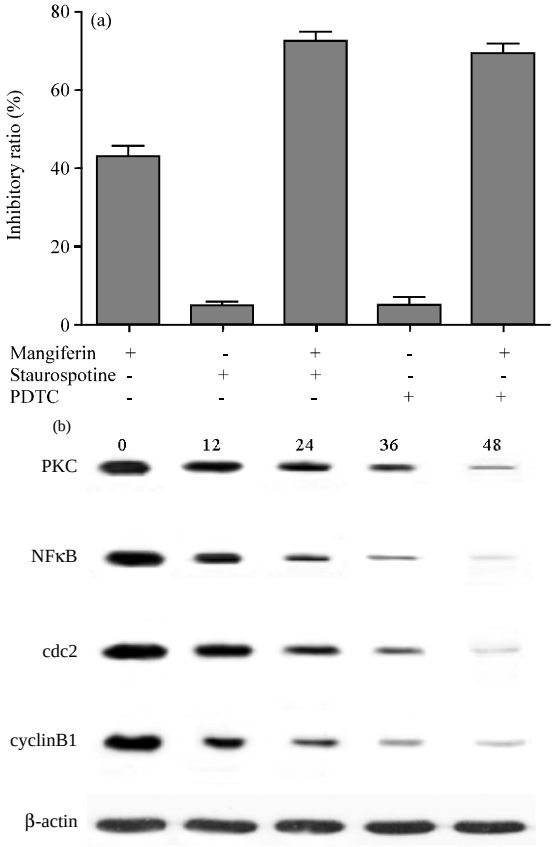

Fig. 3: The molecular mechanism of mangiferin induced G_2/M phase cell cycle arrest and apoptosis; a) MCF-7 cells were pretreated with PKC inhibitor staurospotine and NFκB inhibitor PDTC and mangiferin-induced cell growth inhibitory ratio was measured; b) Cells were treated with 25 μg mL^{-1} mangiferin for various time periods and the expressions of PKC, NFκB, cyclinB1 and cdc2 were detected by western blot analysis. β-actin was used as an equal loading control

resulted in decreased level of cyclin B1 and cdc2. Thus, mangiferin induced G_2/M phase cell cycle arrest through down-regulating the activation of cdc2-cyclin B1.

Tumor volume decreased after mangiferin administration *in vivo*: To further investigate whether mangiferin administration has any effect on the growth of tumors formation, the MCF-7 Xenograft Mouse Model was used in this study. MCF-7 cells were inoculated on the neck of C57BL/6J mice *in vivo* and the administration of mangiferin was lasted for another 2 weeks. Before and after the mangiferin treatment, mouse body weight was measured daily. Subsequently, 5 mice in each group were sacrificed and 5 mice in each group were raised for

survival assay. The subcutaneous tumors were peeled off and weighed. The volume of tumors was determined in three dimensions with vernier calipers and then relative tumor volume was calculated by the equation mentioned above. As shown in Table 1, after 14 days of 100 mg kg^{-1} mangiferin treatment, tumor volume decreased to 0.38±0.29 cm^3, almost reducing 90% compared with blank control group. Similarly, the inhibitory ratio of volume treated with cisplatin also nearly reached to 90%. Meanwhile, the weight of tumor interfered with 100 mg kg^{-1} mangiferin decreased to 0.71±0.13 g, almost reducing 85% while inhibitory ratio of positive control group nearly got to 85% also. It is indicated that mangiferin almost bear similar anti-tumor effects with cisplatin which is an accepted effective anti-tumor agent.

The survival ratio and body weight of MCF-7 cell xenograft mice were interfered with mangiferin: The 5 mice in each group were raised for survival assay. As shown in Fig. 4a, compared with blank control group, mangiferin could prolong life span of MCF-7 cell xenograft mice obviously. Mice without any treatment (blank control) were died within 40 days of MCF-7 inoculation. However, no matter mangiferin or cisplatin (positive control) treatment could significantly increase the lifespan of xenografted mice. Even the low dose of mangiferin treatment (10 mg kg^{-1}) could partially rescue MCF-7 inoculated mice and >60% of mice survived till the end of *in vivo* assay. Meanwhile, the high dose of mangiferin treatment (100 mg kg^{-1}) could significantly increase the life span of xenograft mice, compared with that of the cisplatin treated mice. And there was no significant different between high dose mangiferin treated mice and cisplatin treated mice ($p<0.05$, analyzed by two-way ANOVA). After 7 days (designated as day 6-0) of MCF-7 cells inoculation, tumor was formed and the weight of mice was increased. After treatment with various dosages of mangiferin for 14 days, the weight of mice was decreased obviously (Fig. 4b). Although, there was significant difference between blank control group and all other groups ($p>0.05$, analyzed by t-test), there still existed statistical difference among groups administrated with different dosage of mangiferin ($p<0.05$, analyzed by two-way ANOVA). Meanwhile, the body weight of high dosage mangiferin treated mice almost reached to that of the cisplatin treated mice. Based on the survival ratio and body weight data, it is indicated that mangiferin possesses remarkable anti-tumor activity *in vivo*.

In this study, researchers demonstrated that mangiferin presents growth-inhibitory and apoptosis inducing effects on breast adenocarcinoma MCF-7 cells both *in vitro* and *in vivo*. Firstly, researchers

Table 1: Inhibitory of BFL on MCF-7-bearing mice at the 14th day (n = 10, $p<0.05$)

Groups	Volume/cm^3	Weight/g	Inhibitory ratio of volume (%)	Inhibitory ratio of weight (%)
Blank control	4.23±1.44	7.38±1.65	-	-
100 mg kg^{-1}	0.38±0.29	0.71±0.13	89.4	85.3
50 mg kg^{-1}	0.87±0.16	1.52±0.78	65.0	67.1
10 mg kg^{-1}	1.36±0.21	2.34±1.07	43.2	40.5
Cisplatin (10 mg kg^{-1})	0.21±0.25	0.63±0.09	91.5	85.6

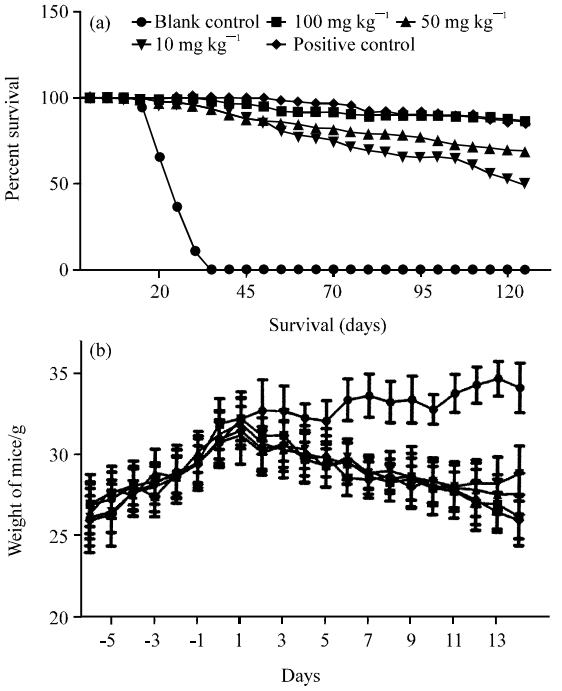

Fig. 4: Mangiferin decrease the volume and weight of subcutaneous tumor mass *in vivo* as well as increase survival ratio and weight of mice; a) Survival curve of MCF-7 cell xenograft mice after various dosage of mangiferin treatment (n = 5, $p<0.05$); b) Weight variety of MCF-7 cell xenograft mice with different dosage of mangiferin treatment and also with control group (n = 5, $p<0.05$)

confirmed that mangiferin could induce breast adenocarcinoma MCF-7 cell arrested in G_2/M phase by downregulating cdc2-cyclinB1 singling pathway. Secondly, mangiferin could induce apoptotic cell death in breast adenocarcinoma MCF-7 cells through inhibiting PKC-NFκB pathway. Finally, mangiferin also bears anti-cancer and apoptosis-inducing effects *in vivo* and it could decrease the volume and weight of subcutaneous tumor mass obviously as well as expand the life span of mice.

Besides, the ratio of G_2/M and sub-G_1 phase cell cycles of MCF-7 cells were markedly enhanced in a

time-dependent manner, indicating mangiferin could induce both G_2/M phase cell cycle arrest and apoptotic cell death in MCF-7 cells. After interfere with 25 μg mL^{-1} drug for 12, 24, 36 and 48 h, the expressions of cdc2 and cyclinB1 were decreased. In eukaryotes, initiaion of cdc2/cyclinB1 complex (M-phase Promoting Factor, MPF) plays important roles in regulating the entry into mitosis of which cdc2 is known as an active sub-unit of the MPF (Chen *et al*., 2008). Overexpression of cdc2/cyclinB1 complex has been found in various tumors. Therefore, suppressing the expression of cdc2-cyclinB1 complex could result in cell growth inhibition (Vairapandi *et al*., 2002). All these results demonstrated that mangiferin could inhibit human breast carcinoma MCF-7 cell proliferation through initiating G_2/M cell cycle arrest by downregulating cdc2-cyclinB1 singling pathway.

Also, mangiferin were illustrated to induce intrinsic mitochondrial-mediated apoptosis in a caspase 3-, 8- and 9-dependent manner in accompany with mitochondrial membrane potential collapse and cytochrome c release from mitochondria into cytosol. Release of cytochrome c appears to be the central event in apoptosis, since it is critical for initiation of caspase family (Goldstein *et al*., 2000). Subsequently, deep exploration of the molecular mechanisms of mangiferin-induced apoptosis indicated that mangiferin initiated apoptotic cell death by suppressing PKC-NFκB pathway. After pretreatment of MCF-7 cells with PKC inhibitor staurospotine and NFκB inhibitor PDTC, mangiferin-induced cytotoxicity was markedly increased, indicating that they play protective role in mangiferin-induced MCF-7 cell apoptosis. Western blot data demonstrated that PKC and NFκB proteins levels were gradually decreased in a time-dependent manner, indicating mangiferin induced apoptosis through suppressing PKC-NFκB signaling pathway in human breast carcinoma MCF-7 cells. Protein kinase C epsilon (PKCε or PKC), an oncogenic kinase is essential in regulating cell survival, mitogenesis and invasion and PKCε is found to be up-regulated in most epithelial cancers including prostate, breast and lung cancer (Reyland, 2007). Strong correlation has been observed between PKC overexpression and NFκB activation and NFκB family of transcription factors plays a crucial role in inflammation as well as controlling the expression of genes involved in cell survival, proliferation, angiogenesis and invasion (Mezzano *et al*., 2004). Thereby, inhibition of PKC-NFκB pathway functions as an effective strategy in cancer treatment.

Previously, numerous reports have demonstrated the anti-cancer effects of mangiferin. Mangiferin can inhibit Tumor Necrosis Factor (TNF)-induced activation of NF-κB in MCF-7 cells (Sarkar *et al*., 2004) and also mangiferina indica extract can suppress MDA-MB-231 cell proliferation through hampering NF-κB pathway MCF-7 cells (Garcia-Rivera *et al*., 2001). Supplementation of mangiferin could enhance the detoxification of enzymes including glutathione transferase, quinone reductase and uridin 5'-diphosphate-glucuronosyl transferase and reduce DNA damage in lung cancer bearing animals (Rajendran *et al*., 2008). In addition, mangiferin was reported to inhibit telomerase activity and induce apoptosis in K562 cells (Cheng *et al*., 2007; Peng *et al*., 2004) and mangiferina indica extract could also initiate G_0/G_1 phase cell cycle arrest (Percival *et al*., 2006).

Expect for *in vitro* anti-cancer activity of mangiferin, mangiferin was also verified to possess anti-tumor effects *in vivo*. Mangiferin could decrease the volume and weight of subcutaneous tumor mass obviously, after 14 days treatment tumor volume and weight decreased nearly 90 and 85%, respectively. Highest dosage of mangiferin injection (100 mg kg^{-1}) almost bears similar anti-tumor effects with cisplatin which serves as an accepted effective anti-tumor agent. All these mentioned-above results indicated that mangiferin bears the capability to inhibit human breast carcinoma MCF-7 cells growth and induce apoptotic cell death both in *in vitro* and *in vivo*.

CONCLUSION

Researchers report for the first time that mangiferin triggers G_2/M phase cell-cycle arrest via downregulating cdc2-cyclinB1 singling pathway and induces apoptotic cell death through inhibiting PKC-NFκB pathway in human breast carcinoma MCF-7 cells. In addition, mangiferin also bears anti-cancer effects *in vivo* and it could decrease the volume and weight of subcutaneous tumor mass obviously as well as expand lifespan. With the molecular mechanisms of mangiferin-induced anti-tumor activities were gradually clarified, traditional Chinese medicine would become potential anti-neoplastic drugs in future cancer therapeutics.

ACKNOWLEDGEMENT

This research was supported in part by National Natural Science Foundation of China (No. 30970643, No. 81173093 and No. J1103518).

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