Protective effects of the ethanolic extract of *Melia toosendan* fruit against colon cancer

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Colorectal cancer is one of the leading causes of death in the world. Plant-derived products have proven to be valuable sources for discovery and development of unique anticancer drugs. In this study, the inhibitory effects of ethanolic extract of *Melia toosendan* fruit (EMTF), a traditional medicine in the Chinese Pharmacopeia were evaluated \textit{in vitro} and \textit{in vivo} against colon cancer. Human colon cancer cells SW480 and murine colorectal adenocarcinoma cells CT26 were used to investigate cell proliferation. The results showed that EMTF inhibited cell proliferation of SW480 and CT26 by promoting apoptosis as indicated by nuclear chromatin condensation and DNA fragmentation. Through increasing mitochondrial membrane permeability and cytochrome c release from mitochondria, EMTF induced caspase-9 activity which further activated caspase-3 and poly(ADP-ribose) polymerase cleavage, leading the tumor cells to apoptosis. The \textit{in vivo} results confirmed reduction of tumor volume and apoptotic effects and the side effects were not induced by EMTF. Therefore, EMTF may be an effective chemotherapeutic agent for colon cancer treatment.

**Keywords:** *Melia toosendan* fruit, Colon cancer, Apoptosis

Colorectal cancer is one of the leading causes of death in the world. In the United States, it has become the second most common cause of cancer-related deaths\textsuperscript{1}. Cancer statistics of 2012 in the United States showed that there were 103,170 estimated new cancer cases of colon cancer and the estimated deaths rate combined with rectum cancers ranked the third of all the cancer induced disease\textsuperscript{2}. Despite recent advances in understanding the carcinogenic processes of colon cancer, the increasing incidence and relatively low success rate of therapy have necessitated the scientific community to seek more effective candidates from different resources for novel therapies.

The potential for using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI)\textsuperscript{3}. Since then a number of new naturally occurring anticancer agents have been discovered. Throughout the history of medicine, plant-derived products have been valuable sources for the discovery and development of unique anticancer drugs. Several therapies used today trace their origins to plants, such as *Vinca* alkaloids, taxanes and camptothecins\textsuperscript{4-6}.

*Melia toosendan* Siebold & Zucc. (Meliaceae) is a common deciduous tree that grows widely from southwestern to eastern China. It has been used as an insecticidal and medicinal plant for a long time in China. The *M. toosendan* fruit (MTF), locally known as “Chuan-lian-zi” or “Jin-ling-zi” is a traditional medicine recorded in the Chinese Pharmacopeia, and it has been used in China for treatment of stomach ache, cholelithiasis and a number of acute and chronic inflammatory conditions, including cholecystitis, gastritis and mastitis, as well as for ascariasis\textsuperscript{7-9}.

Many compounds, including triterpenoids, limonoids, flavonoids and polysaccharides have been
isolated from the fruit and bark of *M. toosendan*. Toosendanin, a triterpenoid derivative extracted from the fruit and bark has shown significant suppressive effects on the proliferation of various human cancer cell lines, including hepatocellular carcinoma cell lines SMMC-7721 (p53+) and Hep3B (p53-), hepatoma cell line BEL-7404, glioblastoma cell line U251, neuroblastoma cell line SH-SY5Y, promyelocytic leukemia cell line HL-60, prostate adenocarcinoma cell line PC3 and histiocytic lymphoma cell line U937. A limonoid compound (28-deacetyl sendanin) isolated from the fruit has also demonstrated cytotoxicity on tumor cells.

However, to date, there has been no report on the antitumor activity of a total ethanolic extract from MTF (EMTF), which could improve understanding of the therapeutic benefits of the whole fruit of *M. toosendan*. Also, there are no systematic evaluations of the effects of MTF on colon cancer. Thus, in this study, we have evaluated the inhibitory effects of EMTF against colon cancer *in vitro* and *in vivo*.

**Materials and Methods**

**Plant material and preparation of EMTF**

The dry fruits of *Melia toosendan* Siebold & Zucc. were purchased from an Oriental Pharmacy (Iksan, Jeonbuk, Korea) and authenticated by Prof. Y C Kim (College of Pharmacy, Wonkwang University, Iksan, Jeonbuk, Korea). A voucher specimen (WP-2009-103) was deposited at the Herbarium of the College of Pharmacy, Wonkwang University. Dried, pulverized fruits (50 g) were extracted under reflux with 70% ethanol for 2 h. The extract was filtered and the filtrate was evaporated in vacuo to give the dried extract (yield 19.0%) as the extract sample for further assays. When used, the EMTF was dissolved in cell culture media.

**Cell culture**

Human colon cancer cell line SW480 and murine colorectal adenocarcinoma cell line CT26 derived from BALB/c mice were obtained from the Korean Cell Line Bank. They were cultured in Roswell Park Memorial Institute medium (RPMI)-1640 supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were incubated at 37°C in a humidified atmosphere with 5% CO₂ and subcultured after trypsinization (Gibco, USA).

**Cell proliferation assay**

The cell proliferation assay was carried out according to the manufacturer's protocol with CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega Corp., USA). Briefly, after 24 h seeding, cells were exposed to different concentrations of EMTF. Following incubation for 24, 48, and 72 h, 20 µl of methanethiosulfonate/phenazine methosulfate solution was added to each well and incubated for 1–1.5 h at 37°C. The absorbance was then read at 490 nm on a microplate reader (Tecan Infinite F200, Switzerland) and the inhibitory rates were calculated as [1 - (OD<sub>treated</sub>/OD<sub>control</sub>) × 100%].

Both the SW480 and CT26 cells were exposed to different concentrations of EMTF respectively for 48 h and cell morphologic changes were assessed using a phase-contrast microscope (CKX41, Olympus, Japan). Thereafter, cells were collected, washed twice with phosphate-buffered saline (PBS), and fixed with 70% ethanol solution at 4°C for at least 2 h. After centrifugation, cell pellets were stained with 50 µg/ml propidium iodide (Pl) and 1 µg/ml RNase A at 4°C for 30 min. The samples were analyzed in a FACScan flow cytometer (Becton Dickinson, USA) by CellQuest software. The cell cycle distributions were analyzed using ModFit LT software. All experiments were repeated and yielded similar results.

**Detection of apoptosis**

SW480 cells were treated with 2.5–20 µg/ml EMTF for 48 h. Cells were fixed with 4% paraformaldehyde and stained with 4’,6-diamidino-2-phenylindole. Stained nuclei were visualized using a fluorescent microscope (IX71, Olympus, Japan). Membrane blebbing, chromatin aggregation and nuclear condensation were used as criteria to identify cells undergoing apoptosis.

**DNA fragmentation analysis**

After treatment of SW480 cells with EMTF for 48 h, DNA fragments were extracted using the method described by Steinfelder et al. Briefly, cells were lysed and suspended in Tris-EDTA buffer (pH 7.5). Then precipitation solution was added, mixed and placed on ice for 15 min. Thereafter, the mixture was spun for 15 min at 14,000 × g and DNA in the supernatant was collected using a miniprep spin column (QIA-prep Spin Miniprep Kit, QIAGEN, Hilden, Germany) and eluted with Tris-EDTA buffer (pH 8.0). The DNA samples were separated by electrophoresis on 1.5% agarose gels containing 1 µg/ml ethidium bromide (EB) and images were visualized under UV light using a gel documentation system (SL-20 Image Visualizer EL Logic 100,
Seoulin Scientific Co.) and captured using the Kodak ML software application.

Measurement of mitochondrial membrane potential (MMP) and release of cytochrome c

After culturing with EMTF for 48 h, SW480 cells were harvested. Changes of MMP were assessed using the mitochondrial probe rhodamine 123. Briefly, after trypsinization, cells were washed twice in PBS and incubated with rhodamine 123 dye at 37°C for 30 min. Cells were then washed twice and re-suspended in PBS for flow cytometric analysis and histograms were analyzed by CellQuest Software. Data were given in percentage of cells with altered MMP.

The method of preparation of cytosolic fractions from cells was modified from previously described method[17]. Briefly, after treatment with EMTF for 48 h, cells were collected and washed with PBS. Cell pellets were resuspended in ice-cold buffer A (20 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.5, 10 mmol/l KCl, 15 mmol/l MgCl₂, 1 mmol/l EDTA, 1 mmol/l ethylene glycol tetraaetic acid, 1 mmol/l dithiothreitol, 5 µg/ml pepstatin A, 10 µg/ml leupeptin, 2 µg/ml aprotinin, and 0.1 mmol/l phenylmethylsulfonyl fluoride), allowed to swell on ice for 30 min, and centrifuged at 4°C for 20 min. The resulting supernatant fraction served as the soluble cytosolic fraction. Samples were analyzed by Western blot using anti-cytochrome c antibody.

Western blot analysis

SW480 cells were cultured and treated with various concentrations of EMTF for 48 h. Proteins were obtained by cell lysis in ice-cold radioimmunoprecipitation assay buffer. Total cell proteins were subjected to electrophoresis on 8-12% polyacrylamide gels, transferred to nitrocellulose membranes and probed with antibodies for caspase-9, caspase-3 and poly(ADP-ribose) polymerase (PARP). The immunoblots were developed and visualized by enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ, USA). β-Actin was used as an internal control.

Tumor syngeneic model in BALB/c mice

The experimental protocol was approved by the Animal Care and Use Committee at the Wonkwang University School of Medicine. Five-weeks-old female BALB/c mice were purchased from Orient Bio. Inc. (Orient Bio. Inc. Kr). They were housed under standardized conditions (20-22 °C, 45-50% relative humidity, 12 h light/12 h dark cycle) and given free access to standard food and water under habituation for 1 week before the experiment. The subcutaneous tumor models were constructed as described previously[18] with some modifications. Cell suspensions of CT26 (2 × 10⁵ 100 µl⁻¹) in serum-free medium were injected into the right flanks of mice. When tumors had reached 4-5 mm in diameter, mice were randomly assigned into control and various treatment groups.

Based on our preliminary experiments on animal experiments, we chose a serie of concentrations of EMTF (5, 10 or 20 mg kg⁻¹) to perform the anticancer experiments in BALB/c mice and the dose of 5-FU was calculated by the conversion formula[19]. Treatments were as follows (n = 10): Control groups were given vehicle alone; EMTF groups were given 5, 10 or 20 mg kg⁻¹ EMTF i.p. once daily (days 1–14) and 5-fluorouracil (5-FU) group was given 30 mg kg⁻¹ 5-FU i.p. once daily (days 1–5). The body weight of all animals was recorded throughout the experimental period as an assessment of drug toxicity. Any mortality during the course of the study was also recorded.

Immunohistochemical analysis of apoptosis in tumor tissues

All mice were euthanized at 15 days after initiation of drug treatment and the tumors were dissected and weighted. Tumor volumes were measured using a vernier caliper and calculated as the formula: tumor volume (mm³) = length × (width)²/2[20]. The tumors were fixed with 10% neutral buffered formalin for 24 h, embedded in paraffin and sectioned into 4 µm slices. Sections were stained with hematoxylin and eosin (H & E). Separate sections containing 3 different areas from each tumor were examined in a blinded manner.

Immunohistochemical analysis of the percentage of apoptotic cells in the excised tumor tissues was carried out by the terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP) in situ nick-end labeling (TUNEL) technique with a TdT-FragEL™ DNA fragmentation detection kit. Paraffin-embedded tumor sections were deparaffinized and treated with 20 µg/ml proteinase K to strip proteins from the nuclei. The sections were incubated with terminal deoxynucleotidyl transferase (TdT) and biotin-dUTP in TdT buffer at 37°C for 1.5 h. Following termination of the reaction and blockade of non-specific binding, sections were
incubated with streptavidin-biotin-peroxidase complex and the sites of peroxidase binding were detected using 3,3′-diaminobenzidine. Thereafter, the sections were counterstained by methyl green and observed under a microscope (CX21, Olympus, Japan).

Statistical analysis

Data were presented as the mean ± S.D. of 3 independent determinations. All experiments were done at least 3 times, with 3 or more independent observations each time. Statistical analysis was performed by a one-way analysis of variance (ANOVA), followed by the Student’s t-test using the Statistical Package for the Social Sciences (SPSS). Significant differences were established at p<0.05.

Results

Inhibitory effects of EMTF on the growth of colon cancer cell lines

Figure 1A and B show the concentration- and time-dependent inhibition of EMTF on colon cancer cell growth. After incubation for 48 h, EMTF significantly reduced the proliferation of SW480 cells by over 50% at concentrations from 20 to 50 µg/ml and the anti-proliferative effect was even more prominent after 72 h (Fig. 1A). The inhibitory effects were also found in CT26 cells and the extract proved to be more effective in the human colorectal carcinoma cells at the concentrations from 20 to 50 µg/ml (Fig. 1B).

EMTF induced apoptosis in colon cancer cell lines

The morphologies of SW480 and CT26 cells treated by EMTF are shown in Fig. 2A. Compared with the adherent and spindle-shaped control cells, the morphology changes of drug-treated cells were notable: the cells lost their adherent phenotype and assumed a circular morphology. In Fig. 2B, the cell cycle distributions of SW480 showed no remarkable changes, except the sub-G1 peaks, which suggested that EMTF could induce apoptosis in SW480 cells, but not cell arrest. The apoptosis could also be found in CT26 cells with more remarkable changes. Percentage of apoptotic cells and distributions of each cell cycle phase are shown in Fig. 2B. Cells treated with various concentrations of EMTF displayed the typical morphology of nuclear chromatin condensation when compared with the control cells (Fig. 3A). The classical laddering pattern of internucleosomal DNA fragmentation was observed (Fig. 3B) and even at the low concentration of 2.5 µg/ml the DNA ladders were obvious, indicating that an irreversible apoptotic death had been induced.

EMTF induced mitochondrial membrane depolarization and cytochrome c release

EMTF strongly induced loss of MMP. The percentage of cells with loss of MMP increased from 5.6% in control cells to 16.5-36.1% after treatment with various concentrations of EMTF for 48 h, respectively (Fig. 4A). EMTF-induced release of cytochrome c from mitochondria to cytosol in SW480 cells is shown in Fig. 4B.

Activation of caspase activity and PARP cleavage by EMTF

Caspases are believed to play a central role in mediating various apoptotic responses. Western blot analyses demonstrated that EMTF induced significant increases in caspase-3 and caspase-9 activation and PARP cleavage (Fig. 4C).

Anti-tumorigenic effects of EMTF in vivo

To further confirm the effect of EMTF on growth of colon cancer cells, an in vivo syngeneic model in mice was used. On the day of sacrifice, EMTF treatments at

![Fig. 1](image-url)—Inhibitory effects of ethanolic extract from M. toosendan fruit EMTF on proliferation of SW480 and CT26 cells [Cells were treated with increasing concentrations of EMTF for 24, 48 or 72 h. Cell viability was determined by methanethiosulfonate/phenazine methosulfate solution assay. Results are expressed as the representation of at least 3 independent experiments. (A) Proliferation of SW480. (B) Proliferation of CT26. The inhibitory rates were calculated as [1 - (OD<sub>treated</sub>/OD<sub>control</sub>) × 100%].)
Fig. 2—Changes in cell cycle after treatment by EMTF [Cells were exposed to EMTF for 48 h and cell morphologic changes were assessed using a phase-contrast microscope. Then the cell cycle was analyzed by flow cytometry. (A) The morphologies (400×) of SW480 and CT26 cells. (B) The DNA histograms of SW480 and CT26. Results expressed as the representation of 3 independent experiments.]

Fig. 3—Apoptosis induced by EMTF in SW480 cells [Nuclear chromatin changes were detected using 4’,6-diamidino-2-phenylindole (DAPI) staining. After exposure to EMTF, cells were collected, fixed with 4% paraformaldehyde, stained with DAPI and visualized using a fluorescent microscope (Bar = 100 μm). (A) Nuclear chromatin. In DNA fragment assays, SW480 cells were exposed to EMTF and DNA was extracted and subjected to electrophoresis. (B) DNA fragments. Results expressed as the representation of at least 3 independent experiments.
the given doses resulted in 20-44.2% rates of tumor suppression, but the inhibition rate of 5-FU group reached up to 81.6% (Fig. 5A and B). Table 1 shows that no significant changes in body weight was observed during the study in either control or EMTF-treated groups and all animals survived until the end of study, whereas a significant reduction of body weight was exhibited by the 5-FU-treated animals, and the reduction began from day 3 and was significant on days 6 and 9.

Tumor tissue pathology of each group was examined after staining with H & E. The control group had no necrotic regions and cells were in close formation with very frequent division of nuclei, whereas EMTF groups showed different grades of necrosis: in the 5 mg kg\(^{-1}\) group, few cells revealed necrosis, while 10 mg kg\(^{-1}\) and 20 mg kg\(^{-1}\) demonstrated massive necrosis, as was observed in the 5-FU group (Fig. 5C). Apoptosis in the tumor tissues in mice transplanted with CT26 cells was detected by TUNEL assay. The results showed that TUNEL-positive cells were rarely observed in tissues of the control group. In contrast, a number of apoptotic cells were observed in the 5 mg kg\(^{-1}\), 10 mg kg\(^{-1}\) and 20 mg kg\(^{-1}\) EMTF groups, as in the 5-FU group (Fig. 5C). These data indicated that EMTF was able to induce tumor cell apoptosis, leading to tumor tissue necrosis and smaller tumor size.

Table 1—Changes in body weight and mortality rate of transplanted CT26 mice treated with EMTF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in body weight (mean ± S.D.) (g)</th>
<th>Mortality</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 3</td>
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<tr>
<td>Control</td>
<td>18.00 ± 0.36</td>
<td>18.32 ± 0.49</td>
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<tr>
<td>5-FU (30 mg/kg)</td>
<td>18.00 ± 0.56</td>
<td>17.86 ± 0.58</td>
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<tr>
<td>EMTF (5 mg/kg)</td>
<td>18.00 ± 0.41</td>
<td>18.07 ± 0.56</td>
</tr>
<tr>
<td>EMTF (10 mg/kg)</td>
<td>18.00 ± 0.49</td>
<td>18.58 ± 0.77</td>
</tr>
<tr>
<td>EMTF (20 mg/kg)</td>
<td>18.00 ± 0.52</td>
<td>18.20 ± 0.58</td>
</tr>
</tbody>
</table>

Mortality was not observed in all groups.

EMTF, ethanolic extract from *M. toosendan*; 5-FU, 5-fluorouracil

Fig. 4—Mechanism of EMTF induced apoptosis in SW480 cells [Changes in mitochondrial membrane potential were assessed by flow cytometry after staining the cells with rhodamine 123. Cytochrome c in cytosolic fractions was isolated and analyzed by Western blot. Expression of apoptosis-associated pathway proteins within SW480 cells following EMTF treatment for 48 h was determined by Western blotting. (A) The percentage of cells with loss of mitochondrial membrane potential corresponding to M1 in A. Results are expressed as the percentage of cells in each phase ± SD of 3 independent experiments. Significantly different from control: ** p<0.01. (B) Cytochrome c in cytosolic fractions. (C) Caspase-9, caspase-3, poly(ADP-ribose) polymerase (PARP), and their cleavage changes. Representative immunoblots from 3 independent experiments are shown. β-Actin was used as the internal control]
Discussion

Over the last decade, basic cancer research has achieved remarkable advances in cancer biology. However, therapeutic applications have been restricted to only a few drugs. For colon cancer therapy, drugs including 5-FU, oxaliplatin, and irinotecan are still the main accepted standard regimens worldwide, in spite of their toxicity. To overcome these limitations, it is necessary to isolate new active medicinal compounds from herbal/natural sources that provide alternative treatment choices for patients.

The present study evaluated the activity of EMTF against colon cancer in vitro and in vivo. Firstly, cell proliferation assays were performed and the results showed EMTF inhibited the colon cancer cell growth in the concentration- and time-dependent manners. Especially after incubation for 48 h, EMTF significantly reduced the proliferation of cells by over 50% at concentrations from 20 to 50 µg/ml and the
anti-proliferative effect was even more prominent after 72 h. The inhibitory effects were also found in CT26 cells and the extract proved to be more effective in the human colorectal carcinoma cells at the concentrations from 20 to 50 μg/ml.

In order to uncover the mechanism involved in EMTF-induced growth suppression, the events that led to inhibition of colon cancer cell proliferation were first evaluated. Apoptosis is a cell suicide program that is essential for the development and maintenance of tissue homeostasis and the elimination of unwanted or damaged cells from multi-cellular organisms. Thus, induction of apoptosis in tumor cells may be considered a protective mechanism against development and progression of cancer. In our present results, during apoptosis, cells lost their adherent and spindle-shaped phenotype and assumed an obvious circular morphology.

To determine a mechanism that might account for the effects of EMTF in colon cancer cells, we investigated its effects on cell cycle distribution. In flow cytometric histograms, apoptotic cells would show DNA fluorescence in sub-diploid regions with DNA content less than those in the G1 phase and the percentage of the sub-G1 peak could subsequently be calculated. As shown in the results (Fig. 2B), the sub-G1 population in treated groups increased with the increments of the EMTF concentration. Moreover, the apoptotic pattern was further revealed by DNA gel electrophoresis. Apoptosis is characterized by a series of morphological changes involving cell shrinkage, chromatin condensation and the formation of apoptotic bodies. These morphological changes were observed in EMTF-treated SW480 cells. Therefore, from all of the aforementioned apoptosis-related experiments, we confirmed that EMTF inhibited colon cancer cell proliferation mainly by inducing characteristic apoptosis.

The mitochondrial pathway is one of the main pathways of apoptosis. The key element in the mitochondrial pathway is the efflux of cytochrome c from the mitochondria to the cytosol. Once cytochrome c is released into the cytosol, cytochrome c together with Apaf-1 activates caspase-9, which then activates caspase-3. In the present study, the rapid loss of MMP and release of cytochrome c were observed in EMTF-treated colon cancer cells. Furthermore, we found that EMTF induced apoptosis by increasing the activation of caspase-9 and -3 activities. Caspase-3, a prevalent caspase that is ultimately responsible for the majority of apoptotic processes, causes the cleavage or degradation of several important substrates, including PARP. Intact PARP can help cells to maintain their viability, but cleavage of PARP facilitates cellular disassembly and it serves as a marker of cells undergoing apoptosis. In the current study, cleavage of PARP was observed to increase after EMTF treatment, suggesting that PARP might answer, at least in part, for the ultimately apoptotic death of colon cancer cells induced by EMTF.

To confirm the antitumor effects of EMTF in vitro, we used a CT26 syngeneic model. On the day of sacrifice, EMTF treatments at the given doses had resulted in 20-44.2% rates of tumor suppression, which indicated that EMTF inhibited CT26 tumor growth significantly. To explain the reasons for EMTF inhibitory effects, apoptosis in the tumor tissues in mice transplanted with CT26 cells was detected by TUNEL assay. The results showed that TUNEL-positive cells were rarely observed in tissues of the control group. In contrast, a number of apoptotic cells were observed in the 5 mg kg⁻¹ EMTF group and increased remarkably in 10 mg kg⁻¹ and 20 mg kg⁻¹ EMTF groups. These data indicated that EMTF was able to induce tumor cell apoptosis, leading to tumor tissue necrosis and smaller tumor size, which further confirmed the corresponding effects of EMTF in vitro.

5-FU, the orthodox chemotherapeutic drug in colon cancer showed an even greater inhibition rate than EMTF with the inhibition rate of 81.6%. However, when the toxicity was taken into consideration, as determined from the changes of the body weight of animals according to Britten, EMTF showed favorable results. In Table 1, there were no significant changes in body weight was observed during the study in either control or EMTF-treated groups, and all animals survived until the end of study, whereas a significant reduction of body weight was exhibited by the 5-FU-treated animals with the poor mental states which were because of serious side effects of 5-FU. Therefore, though EMTF had no comparable effect with 5-FU, it still showed good antitumor effect with no toxicity.

**Conclusion**

In summary, this study clearly demonstrates for the first time that EMTF could inhibit colon cancer cell proliferation and induce apoptosis both in vitro and in vivo. Apoptosis through the mitochondrial
pathway, including loss of the mitochondrial transmembrane potential, cytochrome c release and activation of caspases-9, caspases-3 and PARP is the possible mechanism for the inhibitory effect of EMTF. Therefore, EMTF may be an effective chemotherapeutic agent for colon cancer treatment.

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