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# Induction apoptosis of luteolin in human hepatoma HepG2 cells involving mitochondria translocation of Bax/Bak and activation of JNK

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**Abstract**

Since hepatocellular carcinoma remains a major challenging clinical problem in many parts of the world including Eastern Asia and Southern Africa, it is imperative to develop more effective chemopreventive and chemotherapy agents. Herein, we present an investigation regarding the anticancer potential of luteolin, a natural flavonoid, and the mechanism of its action in human hepatoma HepG2 cells. Using DNA fragmentation assay and nuclear staining assay, it showed that luteolin induced apoptosis of HepG2 cells. Luteolin induced the cytosolic release of cytochrome *c* and activated CPP32. We found that Bax and Bak translocated to mitochondria apparently, whereas Fas ligand (FasL) was unchanged after a treatment with luteolin for 3 h. In addition, it showed that c-Jun $NH_2$-terminal kinase (JNK) was activated after the treatment of luteolin for 3–12 h. Further investigation showed that a specific JNK inhibitor, SP600125, reduced the activation of CPP 32, the mitochondrial translocation of Bax, as well as the cytosolic release of cytochrome *c* that induced by luteolin. Finally, the apoptosis induced by luteolin was suppressed by a pretreatment with SP600125 via evaluating annexin V-FITC binding assay. These data suggest that luteolin induced apoptosis via mechanisms involving mitochondria translocation of Bax/Bak and activation of JNK.


*Keywords:* Apoptosis; Bax; Bak; JNK; Luteolin

## Introduction

Apoptosis, or programmed cell death, has an essential role in controlling cell number in many developmental and physiological settings. Apoptosis is impaired in many human tumors, suggesting that disruption of apoptotic function contributes substantially to the transformation of a normal cell into a tumor cell. Apoptosis is an important phenomenon in chemotherapy-induced tumor-cell killing. Components of the apoptosis signaling cascade including caspases (Nicholson et al., 1995; Talanian et al., 1997) along with several other triggers and regulators such as Fas ligand (FasL) (Sharma et al., 2000) and Bcl-2 family members, are among the most promising targets for pharmacological modulation of cell death (Gross et al., 1999; Kluck et al., 1997).

Accumulating evidence suggests that protein kinases, such as the mitogen-activated protein kinase (MAPK) superfamily (including ERK, JNK, and p38 MAPK) are important regulators of apoptosis (Xia et al., 1995; Tournier et al., 2000). Furthermore, several studies have revealed that activation of c-*jun* $NH_2$-terminal kinase (JNK) is involved in apoptosis induced by various cellular stresses and anti-cancer reagents (Amato et al., 1998; Mizukami et al., 2001; Zanke et al., 1996). However, the mechanism that accounts for the proapoptotic actions of JNK has not yet been elucidated. Recent studies have focused on two different possible mechanisms. First, JNK may cause cell death by regulating the expression of death receptor ligands (Faris et al., 1998).

*Abbreviations:* CPP32, cysteine protease 32 kDa proenzyme; DMSO, dimethyl sulfoxide; JNK, c-Jun $NH_2$-terminal kinase; p38 MAPK, p38 mitogen-activated protein kinase; MTT, (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide; PARP, poly (ADP-ribose) polymerase.

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The second possible mechanism is that JNK may contribute to apoptotic response by regulating the intrinsic cell death pathway involving the mitochondria (Tournier et al., 2000).

Many studies have demonstrated that flavonoids are present in medical and edible plants, and epidemiological studies suggest that flavonoids play an important role in the prevention of carcinogenesis (Birt et al., 2001; Middleton and Kandaswami, 1994). Flavonoids are known to suppress tumor cell growth that is mediated by different type of cell cycle arrest and the induction of apoptosis in several tumor cell lines (Casagrande and Darbon, 2001; Iwashita et al., 2000; Kawaii et al., 1999; Kobayashi et al., 2002; Wang et al., 1999). Luteolin (3′,4′,5,7-tetrahydroxyflavone), an important member of the flavonoid family, is present in various fruits and vegetable. It exhibits a wide spectrum of pharmacological properties including anti-inflammatory and anti-allergic properties (Kimata et al., 2000). Recently, much attention has been paid to its antioxidant properties and to its antiproliferative effects (Lee et al., 2002; Perez-Garcia et al., 2000). It was reported that luteolin has potential for anticancer therapy by inhibiting DNA topoisomerase I and II (Chowdhury et al., 2002; Mittra et al., 2000). In addition, Ko et al. (2002) reported that luteolin demonstrated an effect on the inhibition of proliferation and induction of apoptosis in human myeloid leukemia cells. However, little is known about its biochemical targets.

In recent years, it has become clear that many anticancer drugs induce apoptosis in target cells (Debatin, 2000; Kaufmann and Earnshaw, 2000). Although the participation of apoptosis pathways in drug-induced apoptosis appears to be complex, perturbation of mitochondrial function with opening of permeability transition pores and release of apoptogenic molecules has been observed in most cases of drug-induced apoptosis (Green and Reed, 1998). Consistent with our results, it has been demonstrated that luteolin induces apoptosis of human hepatoma HepG2 cells through increasing cytochrome *c* release from mitochondria. In addition, it has been shown that translocation of Bax and Bak to mitochondria and activation of JNK involved in the action (Wei et al., 2001; Wolter et al., 1997). Thus, the evidence suggests that luteolin possess anticancer potential.

## Materials and methods

*Cell culture.* Human hepatoma HepG2 cells were grown in Dulbecco's modified Eagle's medium (GIBCO BRL, Grand Island, NY) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. The cells were plated at a density of $2 \times 10^4/cm^2$ into tissue-culture dishes and grown at 37 °C in a humidified, 5% $CO_2$ atmosphere in Dulbecco's modified Eagle's medium.

*Assessment of cell viability.* The cells were seeded at $2 \times 10^4$ cells/ml density and incubated with luteolin (0–100 μM) for 24 h. Thereafter the medium was changed and incubated with MTT (0.5 mg/ml) for 4 h. The viable cell number was directly proportional to the production of formazan, which was then solubilized with isopropanol, and measured spectrophotometrically at 563 nm.

*Determination of DNA fragmentation.* Both detached and attached cells were harvested by scraping and centrifugation. After been washed with PBS (with 1 mM $ZnCl_2$), the cells were resuspended in 0.5 ml lysis buffer (0.5% Triton X-100, 20 mM EDTA, and 5 mM Tris; pH = 8.0 ) for 45 min. Fragmented DNA in the supernatant fraction after centrifugation at 14000 rpm was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) and once with chloroform; and then it was precipitated with ethanol and 5 M NaCl overnight at −20 °C. The DNA pellet was washed once with 70% ethanol, resuspended in Tris-EDTA buffer (pH 8.0), and treated with 100 μg/ml RNase A for 2 h at 56 °C. After quantitative analysis of DNA content by spectrophotometry (260 nm), an equal amount of DNA was electrophoresed in horizontal agarose gel (1.8%) performing at 1.5 V/cm for 3 h. DNA in gel was visualized under UV light after staining with ethidium bromide (0.5 mg/ml).

*DAPI staining.* Changes in cell morphology characteristic of apoptosis were examined by fluorescence microscopy of DAPI-stained cells. The monolayer of cells was washed in PBS and fixed with 4% paraformaldehyde for 30 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for three times and incubated with 1μg/ml of 4,6-diamidino-2-phenylindole (DAPI) for 30 min, and, then, washed with PBS for three times. The apoptotic nuclei (intensely stained, fragmented nuclei and condensed chromatin) were examined under 400× magnification using a fluorescent microscope with a 340/380 nm excitation filter and scored according to the percentage from 200 to 300 cells/sample by at least two investigators.

*Release of cytochrome c and translocation of Bax, Bak.* The basic methodology for the preparation of mitochondria and cytosol fractions was as described as Tang et al. (1998). Cells ($3 \times 10^6$) were harvested and washed with ice-cold PBS at the end of treatment. The cell pellet was resuspended in 500 μl of buffer A (20 mM HEPES–KOH, pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM sodium EDTA, 1 mM leupeptin, 1 μg/ml pepstatin A, and 1 μg/ml chymostatin), and homogenized in the same buffer with a Pyrex glass homogenizer using a type B pestle (40 strokes). The homogenate was then centrifuged at 1000 × *g* for 10 min at 4 °C. The resulting supernatant was subjected to another centrifugation at 10000 × *g* for 20 min at 4 °C. The resulting supernatant contained the cytosolic fraction, and the pellets contained the enriched mitochondria fraction. Pellets containing mitochondria were treated with lysis buffer (1x PBS, 1% NP40, 0.5% sodium deoxycho-

late, 0.1% SDS, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 10 μg/ml phenylmethylsulfonyl fluoride, 2 mM sodium orthovanadate, and 1 mM DTT) and were incubated on ice for 20 min. Then the lysate was centrifuged at 10 000 × *g* for 10 min at 4 °C, and the resulting supernatant was kept as the solubilized enriched mitochondria fraction. The cytosolic fraction was further centrifuged at 100 000 × *g* (4 °C for 1 h) to generate cytosol. The protein concentration was determined by a Bio-rad protein assay kit, and 25 μg of each fraction was loaded onto a 15% SDS-PAGE. Proteins were then blotted onto a NC membrane (Sartorious), and the membranes were reacted with the primary antibodies (anti-Bax, anti-Bak, and anti-cytochrome *c* and anti-β-actin as internal control). The secondary antibody was a peroxidase-conjugated goat antimouse antibody. After binding, the bands were revealed by enhanced chemiluminescence using the ECL commercial kit. Three independent experiments were conducted and showed the same pattern of changes in the levels of Bax, Bak and cytochrome *c*, a representative one shown here.

*Preparation of total cell extracts and immunoblots analysis.* Cells were plated onto 15 $cm^2$ dishes at a density of $2 \times 10^5$ cells/ml with or without luteolin (50 μM, 0–48 h) and harvested. To prepare the whole-cell extract, cells were washed with PBS plus zinc ion (1 mM) and suspended in a lysis buffer (50 mM Tris, 5 mM EDTA, 150 mM NaCl, 1% NP 40, 0.5% deoxycholic acid, 1 mM sodium orthovanadate, 81 μg/ml aprotinine, 170 μg/ml leupeptin, 100 μg/ml PMSF; pH = 7.5). After mixing for 30 min at 4 °C, the mixtures were centrifuged (10 000 × *g*) for 10 min, and the supernatants were collected as whole-cell extracts. The protein content was determined with Bio-Rad protein assay reagent using bovine serum albumin as a standard. The ECL Western blotting was performed as follows. An equal protein content of total cell lysates from control and luteolin-treated samples was resolved on 10–12% SDS-PAGE gels along with prestained protein molecular weight standard (Bio-Rad). Proteins were then blotted onto NC membrane (Sartorious), and reacted with the primary antibodies (anti-CPP32 and anti-PARP from Transduction Laboratories; anti-FasL, anti-Bcl-2 and anti-Bid from New England Biolabs; anti-phospho-ERKs ($Thr^{183}/Tyr^{185}$), anti-phospho-JNKs ($Thr^{183}/Tyr^{185}$), and anti-phospho-p38 ($Thr^{180}/Tyr^{182}$) from Promega Inc.; as well as anti-β-actin from Sigma as an internal control). The secondary antibody was a peroxidase-conjugated goat antimouse antibody. After binding, the bands were revealed by enhanced chemiluminescence using the ECL commercial kit. All the data are the representative of three independent experiments, respectively.

*Determination of the effects of MAPKs inhibitors.* Hep G2 cells were pretreated with 20 μM of p38 MAPK inhibitor, SB203580, or JNK inhibitor, SP600125 for 1 h, followed by a treatment with 80 μM luteolin for 12 h to analyze CPP32 and for 6 h to analyze Bax and cytochrome *c*, as previously described. In addition, the effect of JNK inhibitor on the luteolin-induced apoptosis was assessed using annexin V-fluorescein isothiocyanate (FITC)-binding assay. Briefly, HepG2 cells were pretreated with SP600125 for 1 h, followed by a treatment with 80 μM luteolin for 18 h, and, then 100 μl of diluted annexin V-FITC solution was added. The apoptotic cells (V+/PI−) were measured by the fluorescence-activate cell sorter analysis in a FACScan analyzer (Becton Dickinson). The data were a representative of three independent experiments.

## Results

### *Luteolin-induced apoptotic death*

Cytotoxicity of luteolin on human hepatoma HepG2 cells was measured by MTT assay. The cell viability curve showed that luteolin presented a dose-dependent effect on the cytotoxicity of HepG2 cells (data not shown) with IC50 near to 80 μM. Thereafter, we used the concentrations of 0–80 μM in the following investigations. In Fig. 1A, the exposure of 40 and 80 μM of luteolin for 18 h induced internucleosomal DNA fragmentation in HepG2 cells, clearly showing the characteristic ladder of oligonucleosomal DNA fragments. In addition, DAPI staining was used to assess the percentage of apoptotic cells with condensed and fragmented chromatin over the control HepG2 cells, and the representative photographs are shown in Fig. 1B. The results indicated that luteolin induced apoptosis in a dose-dependent manner in Fig. 1C. Luteolin treatment (80 μM) for 24 h exhibited 44% of apoptosis in HepG2 cells.

### *Effect of luteolin on CPP32, PARP, and cytochrome c*

The CPP32/caspase-3 (like) protease plays an essential role in executing apoptosis and is synthesized as a proenzyme cleaved into active heterodimers (20 and 10 kDa) when cells undergo apoptosis (Nicholson et al., 1995). We then examined the effect of luteolin on CPP32. As shown in Fig. 2A, cellular CPP32 was activated in response to 40 and 80 μM luteolin treatment as assessed by the decline in the 32 kDa band from 6 h after treatment. One of the substrates of CPP32 during apoptosis is PARP (Tewari et al., 1995), an enzyme that appears to be involved in DNA repair, genome surveillance of integrity resulted from environmental stress. The proteolytic cleavage of PARP is used as an indicator of CPP32 activation. The results showed that PARP was cleaved 6 h after luteolin treatment (Fig. 2A). Since cytochrome *c* is reported to be involved in the activation of the caspases, we examined the level of cytochrome *c* in the cytosol by Western blot analysis. This protein was detectable in the cytosol 3 h after the treatment of luteolin (Fig. 2B).

*Effect of luteolin on FasL and Bcl-2 family*

It has been observed that a variety of chemotherapeutic agents induce apoptosis in susceptible cell types by upregulating FasL (Kaufmann and Earnshaw, 2000). Below, we examine the effect of luteolin on the protein expression of FasL in HepG2 cells. There was little change noted after a treatment with or without luteolin (Fig. 3A). In addition, Bcl-2 family proteins play a pivotal role in controlling cell life and death. Recently, investigations of the Bcl-2 gene family have shown a complex network-regulating apoptosis (Kroemer, 1997). Three subfamilies of Bcl-2 protein have been identified as playing important roles in the apoptotic response. The Bcl-2 subfamily (e.g., Bcl-2 and Bcl-$X_L$) functions to inhibit apoptosis, whereas the Bax subfamily (e.g., Bax, Bak, and Bcl-$X_s$) and the BH3-only subfamily (Bid, Bad, and several others) promote apoptosis (Harris and Thompson, 2000). Bcl-2 is an integral membrane protein that prevents apoptosis in numerous biological systems. The expression of Bcl-2 is inversely correlated

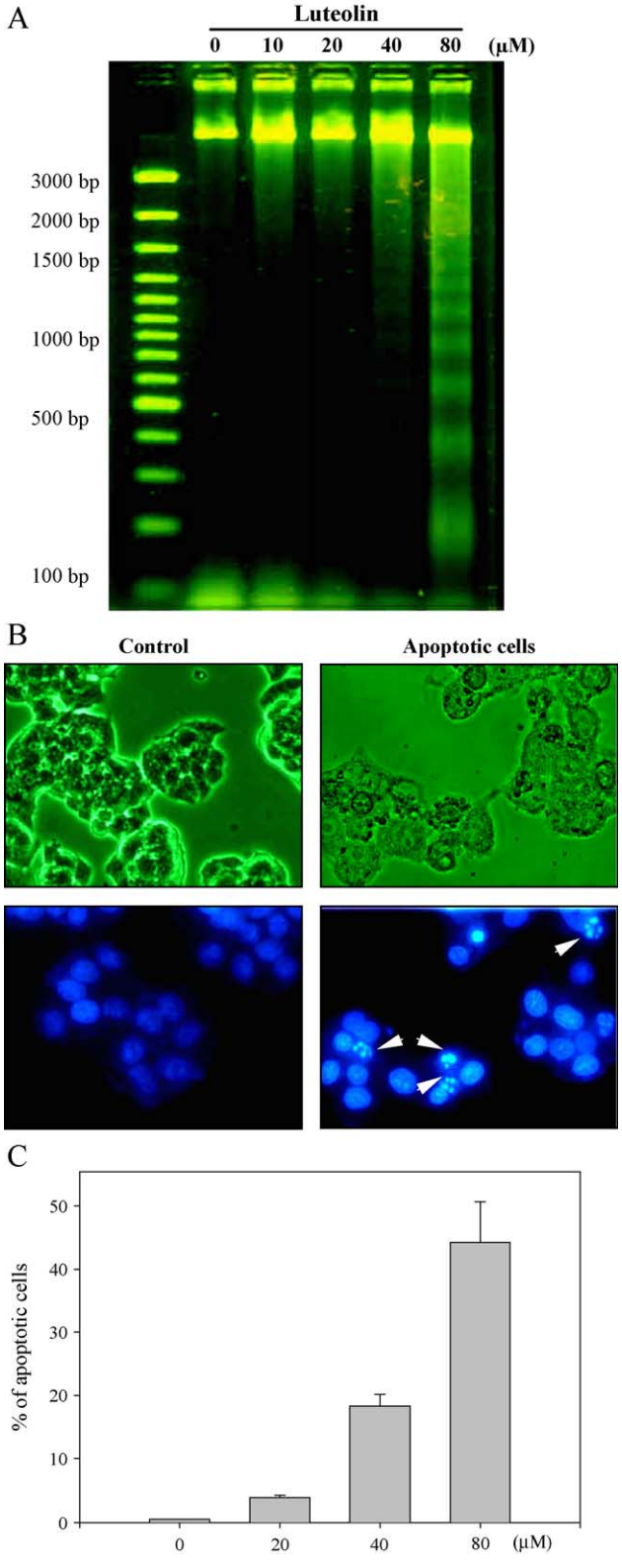


Fig. 1. Luteolin-induced apoptosis in human hepatoma HepG2 cells. (A) Agarose gel electrophoresis of DNA from luteolin-treated HepG2 cells. Cells were treated with various concentration of luteolin for 18 h, and assessed for DNA fragmentation assay as described in Materials and methods. (B) Changes in nuclei by DAPI staining. HepG2 cells treated with 80 μM luteolin or 0.2% DMSO (as control) for 24 h were fixed, permeabilized, and stained with DAPI, and then observed by phase contrast microscopy (upper panels) and fluorescence microscopy (lower panels). Arrows indicate condensed and fragmented nuclei. (C) Quantitation of apoptotic cells was done by DAPI staining. HepG2 cells treated with various concentrations of luteolin or 0.2% DMSO for 24 h were fixed, and stained with DAPI. Apoptotic cells were counted under fluorescence microscopy as described in Materials and methods.

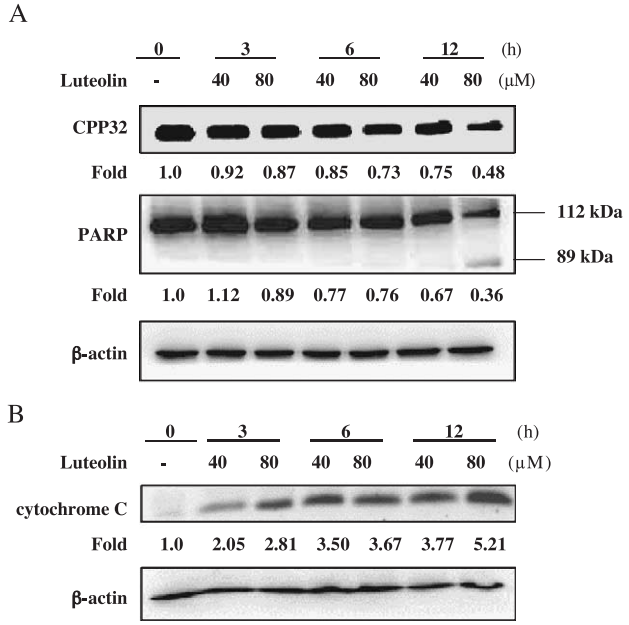


Fig. 2. Effect of luteolin on CPP32, PARP and cytochrome *c*. (A) Effect of luteolin on CPP32 and PARP. Equal proteins of total cell lysates from HepG2 cells with or without treatment of luteolin for 3, 6, and 12 h were analyzed by 12% SDS-PAGE for CPP32 or 10% for PARP. Subsequently, they were immunoblotted with antibodies against CPP32 PARP, and β-actin that served as internal control. (B) Luteolin-induced release of cytochrome *c*. Equal amounts of cytosolic protein were separated by a 15% SDS-PAGE, and subsequently, immunoblotted with antibodies against cytochrome *c* and β-actin.

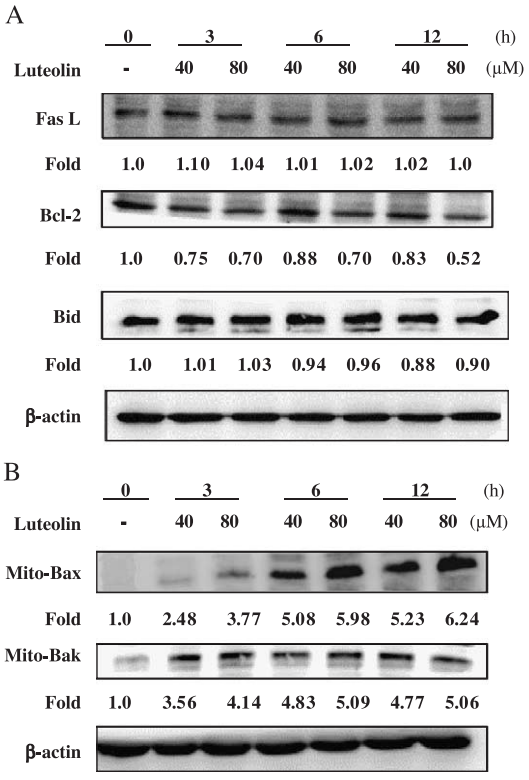


Fig. 3. Effect of luteolin on FasL and Bcl-2 family. (A) Effect of luteolin on FasL, Bcl-2, and Bid expression. Total cell lysates of HepG2 cells treated with or without luteolin for the indicated time were analyzed by SDS-PAGE and, subsequently, immunoblotted with antisera against FasL, Bcl-2, Bid, and β-actin. Three independent experiments were conducted and showed the same pattern of changes in the levels of FasL, Bcl-2, and Bid, a representative one shown here. (B) Effect of luteolin on translocation of Bax and Bak. Mitochondrial fractions of HepG2 cells treated with or without luteolin for indicated times were analyzed by SDS-PAGE and subsequently immunoblotted with antisera against Bax, Bak, and β-actin.

with the susceptibility of cells to apoptosis. The proapoptotic Bcl-2 family members Bax and Bak have been observed to translocate from the cytoplasm to the outer mitochondrial membrane, where they oligomerize to form pores and mediate cytochrome *c* release (Nechushtan et al., 1999). In addition, the fragment of Bid (t-Bid) resulting from caspase-8- and myristoylation-mediated cleavage during Fas-induced apoptosis facilitates oligomerization of Bax and Bak (Wei et al., 2000). Therefore, we examined the cellular levels of Bcl-2 and Bid after treatment of HepG2 cells with luteolin. It was found that the protein expression of Bcl-2 was decreased to 70% after treated with 80 μM of luteolin for 6 h (Fig. 3A), and Bid showed only a little decrease. For the Western immunoblotting analysis of mitochondrial fractions, it showed that after the treatment of luteolin for 3 h, the translocation of Bax to the mitochondria increased to 3.77 fold and Bak to 4.14 fold of that of control (Fig. 3B).

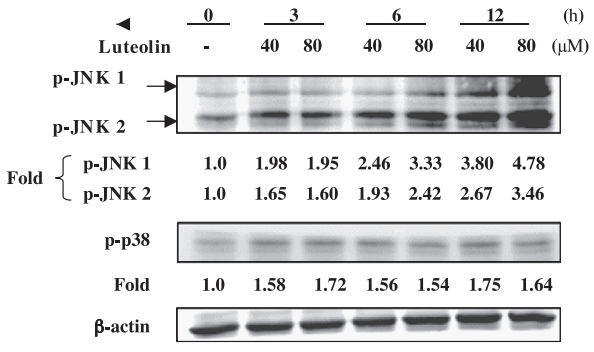


Fig. 4. Effect of luteolin on JNK and p38MAPK activation. Total cell lysates of HepG2 cells treated with or without luteolin for indicated times were extracted, and the phosphorylated JNK and p38 MAPK (active form) were immunodetected as described previously.

*Effect of luteolin on JNK and p38 MAPK*

Fig. 3B shows that luteolin clearly induced the translocation of Bak and Bax to the mitochondria. Previous evidence demonstrated that the activation of p38 MAPK by T cell receptor (TCR) (Yoshino et al., 2001) and the activation of JNK by vitamin E succinate (Yu et al., 2003) involved the translocation of Bax to the mitochondria. Thus, we investigated the effect of luteolin on JNK and p38 MAPK. The result showed that there was an activation of JNK by luteolin in a time-dependent manner and a lesser activation of p38MAPK after the treatment of luteolin for 3–12 h (Fig. 4).

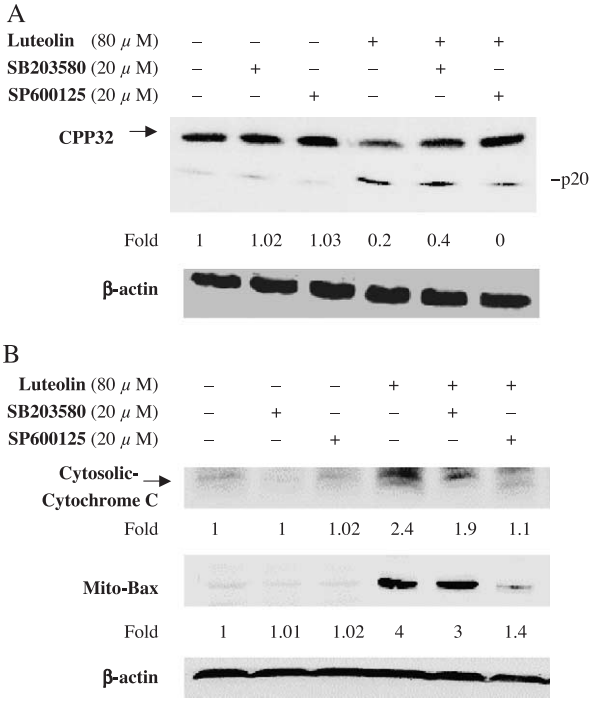


Fig. 5. Involvement of JNK in luteolin-induced CPP32 activation, translocation of Bax, and release of cytochrome *c*. HepG2 cells were pretreated with or without p38 inhibitor, SB203580 or JNK inhibitor, SP600125, for 1 h then treated with luteolin for 12 h to analyze CPP32 or 6 h to analyze Bax and cytochrome *c*, as described in Materials and methods.

*Involvement of JNK in luteolin-induced apoptosis*

Fig. 5 shows that a pretreatment of the JNK inhibitor, SP600125, reduced the activation of CPP32 and the translocation of Bax to the mitochondria, as well as the release of cytochrome *c* to the cytosol induced by luteolin in Hep G2 cells; while the p38 inhibitor, SB203580, showed only minor effect. Fig. 6 presents that the JNK inhibitor decreased the luteolin-induced apoptosis from 41% to 20%; however, the MEK inhibitor, PD98059, had completely no effect on the luteolin-induced apoptosis.

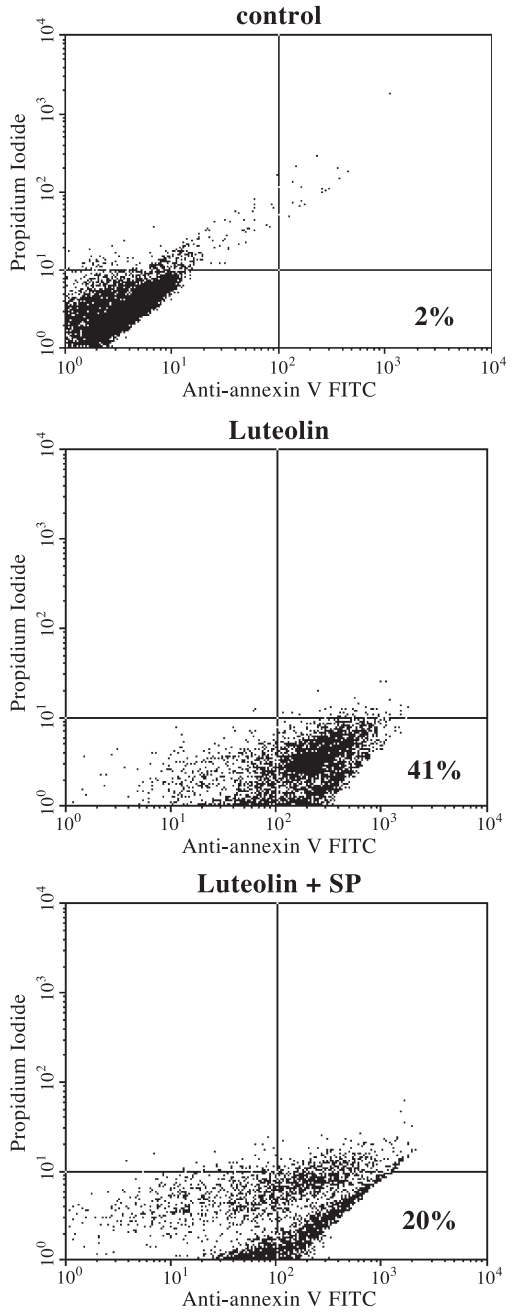


Fig. 6. Effect of JNK inhibitor in luteolin-induced apoptosis. HepG2 cells were pretreated with or without JNK inhibitor (SP600125) for 1 h followed by the treatment with 80 μM of luteolin for 18 h. The cells were stained with FITC-conjugated annexin V and PI for flow cytometry.

## Discussion

Epidemiological studies have consistently shown that flavonoids might play a prominent role in cancer prevention, since these compounds are found in numerous plants that are associated with a reduced cancer rate (Birt et al., 2001). It was reported that luteolin affects the initiation and promotion phases of the carcinogenic process and enhances the detoxification process such as increasing the activity of GST (Elangovan et al., 1994). The observed antiproliferation properties of the 27 citrus flavonoids against several tumor and normal human cell lines, luteolin was found to be the most potent of all (Yamashita and Kwanishi, 2000). Ko et al. (2002) reported that luteolin induced apoptosis in human myeloid leukemia cells. Lee et al. (2002) demonstrated that luteolin inhibited significantly the growth of pancreatic tumor cells and induced apoptosis by modulating EGFR kinase. Our result showed that luteolin induced apoptosis via JNK activation in human hepatoma cells. In addition, it was reported that luteolin inhibited DNA topoisomerase II of *Leishmania* leading to apoptosis (Mittra et al., 2000) while it revealed a strong clastogenic effect and topo II poisoning in V79 cells (Snyder and Gillies, 2002). Recently, luteolin was reported to be similar to camptothecin as an inhibitor of topoisomerase I (Chowdhury et al., 2002). Collectively, evidences suggest that luteolin may be used as a potential anticancer agent, although the relevance of the observation to cancer prevention and chemotherapy remains to be elucidated.

Cell death is as important as cell proliferation in regulating development and homeostasis in multicellular organisms. Physiological cell death is usually mediated through apoptosis, which is positively or negatively regulated by various extracellular factors. It has been demonstrated that a wide range of anticancer and chemopreventive agents induce apoptosis in malignant cells in vitro (Dong, 2003; Hu and Kavanagh, 2003). Apoptosis is characterized by cell shrinkage, chromatin condensation, DNA fragmentation, and the activation of specific cysteine proteases known as caspases. In many apoptotic responses, mitochondria play a major role in coordinating caspases activation through the release of apoptogenic factors, such as cytochrome *c*, AIF (apoptosis-inducing factor) and endonuclease G (Green and Reed, 1998; Li et al., 2001). Members of Bcl-2 family control the release of these proteins. The anti-apoptotic members of the Bcl-2 family, such as Bcl-2 or Bcl-$X_L$, inhibit the efflux of these proteins; whereas the pro-apoptotic members, Bax or Bak, trigger their release (Gross et al., 1999). In this study, we first demonstrated that the luteolin-induced apoptosis of HepG2 cells involved the mitochondria translocation of Bax and Bak (Fig. 3B). In addition, the expression of Bcl-2 was a little decreased after the treatment of luteolin.

The JNK family belongs to the mitogen-activated protein kinase (MAPK) superfamily, which also includes the extracellular signal-regulated kinases (ERKs) and the p38 MAPK family. Although there are exceptions, a bulk of evidence suggests that the activation of ERK pathway increases the cell death threshold in an unknown way (Ishikawa and Kitamura, 1999). Conversely, the activation of JNK and p38 kinase cascades are generally associated with an enhanced activation of the apoptotic program (Ichijo et al., 1997). Consistent with these reports, our results revealed that luteolin activated JNK and p38 kinase in HepG2 cells (Fig. 4) accompany an induction of apoptosis. One possible mechanism accounting for the proapoptotic action of JNK is the regulation of the expression of death receptor ligands. However, we did not find any influence of luteolin on the FasL of HepG2 cells (Fig. 3A). According to our investigation JNK inhibitor reduced the activation of CPP32 and the mitochondrial translocation of Bax and Bak, as well as the cytosolic release of cytochrome *c* induced by luteolin (Fig. 5); and it decreased the luteolin-induced apoptosis (Fig. 6). These data demonstrate that JNK plays an important role in the luteolin-induced apoptosis in HepG2 cells.

Taken together, these data demonstrated that the mitochondrial translocation of Bax/Bak and the activation of JNK were critical events in the luteolin-induced apoptosis of human hepatoma HepG2 cells. We suggest that luteolin possesses anticancer potential.

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