Genipin Induced Apoptosis Associated with Activation of the c-Jun NH₂-Terminal Kinase and p53 Protein in HeLa Cells

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Genipin is a metabolite of geniposide isolated from an extract of *Gardenia fructus*. Some observations suggested that genipin could induce cell apoptosis in hepatoma cells and PC3 human prostate cancer cells. However, the effects of genipin on HeLa human cervical carcinoma cells are still unknown. In this study, we provided evidences that genipin induced the death of HeLa cells through apoptotic pathway in a dose-dependent manner. Genipin could remarkably induce cytotoxicity in HeLa cells and inhibit its proliferation. Induction of the apoptosis by genipin was confirmed by analysis of DNA fragmentation and induction of sub-G₁ peak through flow cytometry. The results also showed that genipin-treated HeLa cells cycle was arrested at G₁ phase. Western blot analysis revealed that the phosphorylated c-Jun NH₂-terminal kinase (JNK) protein, phospho-Jun protein, p53 protein and bax protein significantly increased in a dose-dependent manner after treatment of genipin for 24 h, and to our knowledge, the activation of JNK maybe result in the increase of the p53 protein level, and the increase of the p53 protein led to the accumulation of bax protein, bax protein further induced cell apoptotic death eventually. Taken all these together, it is possible to develop genipin as an anti-cancer drug.

Key words genipin; apoptosis; c-Jun NH₂-terminal kinase; p53; G₁ phase; HeLa cell

Geniposide is one of the main irioid-glyco compounds isolated from *Gardenia* fruits which have been used as Chinese medicine for the treatment of hepatotic disorders and inflammatory diseases for many years.¹⁾ Geniposide is hydrolyzed to the aglycone genipin by bacterial enzymes, β -D-glycosidases in the intestines and the liver.^{2,3)} Genipin has been shown to possess anti-inflammatory, anti-diabetic, anti-thrombotic, anti-oxidative, anti-angiogenic and neurotrophic activities.^{4–7)} Recently, genipin has been reported to induce apoptosis in some cancer cells, such as FaO rat hepatoma cells, human hepatocarcinoma Hep3B cells and PC3 human prostate cancer cells, and c-Jun NH₂-terminal kinase (JNK) contributes to the apoptosis.^{8,9)} Therefore we studied whether the same pattern would occur in genipin-induced HeLa cells.

Apoptosis is a programmed cell death progress. It is characterized by a process in which catabolic enzymes degrade essential macromolecules leading to a characteristic biochemical and morphological death phenotype including cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation, and formation of apoptotic bodies.^{10,11)} Apoptosis is very important to develop and maintain homeostasis during cell growth and elimination of damaged cells in multicellular organisms.⁹⁾

JNK, which is one of mitogen-activated protein kinases family (MAPKs), also known as stress-activated protein kinase (SAPK), is an important regulatory protein through which various extracellular stimuli and stresses can be transduced into intracellular events. JNK is responsible for the phosphorylation of a variety of proteins including downstream kinases and transcription factor such as c-Jun.¹²⁾ Activated JNK specifically catalyzes and phosphorylates c-Jun on its N-terminal transactivation domain at Ser63 and Ser73.^{13–15)} The expression of phosphorylated JNK and phosphorylated c-Jun was examined in our study. The tumor suppressor and transcription factor p53 is a key modulator of cellular stress responses, and activation of p53 can trigger apoptosis in many cell types.¹⁶⁾ The level of p53 protein is

kept low in normal cells but increase in injured cells.¹⁷⁾ Over-expression of p53 inhibits cellular proliferation.¹⁸⁾ Activation of p53 regulates lots of gene, including the p21^{WAF1/CIP1} which is reluctant to cell cycle arrest at G₁ phase. Genipin was shown to induce HeLa cell cycle arrest at G₁ phase in our study. Therefore we analyzed the level of p53 protein. In addition, bax which is a member of bcl-2 family, also plays a crucial role in apoptosis as a pro-apoptotic protein. The accumulation of bax protein can subsequently induce the release of cytochrome *c* and result in the mitochondria-dependent apoptosis and cell apoptotic death eventually.¹⁹⁾

Above all, the effect of genipin on HeLa cells and whether the act is in a dose manner haven't been reported. In the present study, we investigated that cell death by genipin in HeLa cells was due to apoptosis by analysis of cell viability, DNA fragmentation and induction of sub-G₁ peak. We also evaluated the cell cycle arrest by genipin. Moreover, to study the apoptotic mechanism in genipin-treated HeLa cells, we examined the protein levels of phosphorylated JNK, phospho-Jun, p53 and bax.

MATERIALS AND METHODS

Chemicals Antibodies, anti- β -actin and anti-phospho-Jun were obtained from Cell Signal Technology (U.S.A.). Antibodies, anti-phospho-JNK and anti-p53 were from Millipore (U.S.A.). Bax antibody was from Santa Cruz (U.S.A.). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Amresco (U.S.A.). Peroxidase-labeled sheep anti-rabbit and sheep anti-mouse immunoglobulin were from ZSGB-BIO (Beijing, China). Enhanced chemiluminescence (ECL) Western blot detection system was purchased from Millipore Corp. (U.S.A.). Genipin powder was from Haikang Biotechnology Ltd. (Chengdu, China), genipin powder was dissolved in ddH₂O at 0.05 M as a stock solution. All of the stock solutions were kept at 4 or –20 °C. All other chemicals were analytical pure.

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Cell Culture HeLa cells were from transplantation and immunization lab., West China Hospital of Sichuan University (China). These cells were maintained at 37 °C in humidified air atmosphere with 5% CO₂. The culture medium for these cells was consisted of RPMI 1640 medium (GIBCO, U.S.A.) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, HyClone, China), and antibiotics (100 units/ml of penicillin and 100 µg/ml of streptomycin) (Solarbio, Beijing, China). For treatment, HeLa cells were trypsinized, seeding at a density of about 2×10⁵ cells/ml per flask. All experiments were performed in plastic tissue culture flasks, dishes, or plates.

Cell Number Assay HeLa cells were seeded at a density of 0.8×10⁶ per 100 mm plastic dish. Then the cells were treated with different concentrations of genipin (at 45, 90, 135 µg/ml) for 24 h and 48 h. At the end of the incubation period, cells were trypsinized and total cell number was counted by a hemocytometer.

In Vitro Assay for Cytotoxic Activity (MTT Assay) HeLa cells were seeded in 96-well plates at a density of 1×10⁴ cells/well. After the cells were treated with different concentrations of genipin (at 45, 90, 135 µg/ml) for 24 h, the medium was removed, followed by the cells were washed with phosphate buffered saline (PBS) twice, then 20 µl MTT (5 mg/ml) was added into each well and the cells were incubated at 37 °C for 4 h. Finally, 150 µl dimethyl sulfoxide (DMSO) was added. The absorbance at 492 nm and 630 nm was measured using MULTISKAN MK3 (Thermo, U.S.A.). The cell viability (% of control) was calculated by the following equation:

$$\text{cell viability (\% of control)} = A_{\text{treated}} / A_{\text{control}} \times 100\%$$

Cytomorphology HeLa cells were incubated with indicated concentrations (at 45, 67.5, 90 µg/ml) of genipin for 24 h at 37 °C, then were directly observed under inverted phase-contrast microscopy (Leica DMIRB, German).

Determination of DNA Fragmentation Cells cultured on a 100 mm plastic dish were treated with various concentrations of genipin (at 45, 90, 135 µg/ml) for 24 h. The untreated and genipin-treated cells were harvested and washed with cold PBS twice. Fragmented DNA was extracted according to instructions supplied by the manufacturer of DNA Ladder kit (Beyotime, Shanghai, China), and electrophoresed in 1.5% agarose gel at 50 V for 2.5 h and stained with ethidium bromide (EB). Finally the gel was photographed under ultraviolet light.

Assessment of Flow-Cytometry After cells were treated with different concentrations of genipin (at 45, 90 µg/ml) for 24 h, treated and untreated cells were harvested and washed twice with PBS. Cells were fixed by 70% cold ethanol at 4 °C for 1 h, followed by staining with propidium iodide (PI) at 4 °C. DNA-bound PI fluorescence was measured with 15 mW air-cooled argon ion laser at 488 nm as excitation sources, using an EPICS ELITE ESP flow cytometer (Beckman Coulter, U.S.A.). Analysis of cell cycle and apoptosis was performed with 10000 events for each sample and the data were analyzed by using Coulter Elite 4.5 Multicycle software.²⁰⁾

Western Blot Detection of Phospho-JNK, Phospho-Jun, p53 and Bax The untreated and genipin-treated cells were harvested and washed with cold PBS. Whole-cell extracts

were obtained in lysis buffer (250 mM NaCl, 10 mM Tris, pH 7.4, 1 mM ethylene diamine tetraacetic acid (EDTA), 1% Triton X-100, 1 mM phenylmethanesulfonyl fluoride (PMSF)), followed by centrifugation (12000×g, 30 min). The amounts of protein were normalized by Bradford method.²¹⁾ Equal amounts of total protein (100 µg/lane) were subjected to 12.5% polyacrylamide gels, and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, U.S.A.). After blocking with 5% bovine serum albumin with 0.05% Tween in triethanolamine-buffered saline solution (TBS) (100 mM Tris-HCl, pH 7.5, 0.9% NaCl) the membranes were, respectively, incubated with primary and secondary antibodies. Then, the membranes were washed with TBS containing 0.05% Tween three times. Bands were visualized by using Bio-Rad ChemiDoc XRS imager (Hercules, CA, U.S.A.) with the ECL Western blot detection system. The densities of bands were determined by densitometry using Quantity One software (Bio-Rad, U.S.A.).

Statistic Analysis The significance of the difference between the treated and untreated groups was determined with the Student's *t*-test. The results are presented as mean±S.D. of three independent experiments. The differences were considered significant at *p*<0.05.

RESULTS

Effect of Genipin on Cell Proliferation of HeLa Cells

To investigate whether genipin could inhibit the growth of HeLa cells, we counted the cell number by a hemocytometer. After HeLa cells were incubated with indicated concentrations of genipin (at 45, 90, 135 µg/ml) for 24 h and 48 h. The growth of HeLa cells was significantly inhibited with the treatment of genipin (Fig. 1A). Higher concentrations of genipin (at 90, 135 µg/ml) almost completely blocked the cell proliferation ability.

Effect of Genipin on Cell Viability of HeLa Cells Cell viability was measured using MTT assay. The viable cell number/well is directly proportional to the production of formazan,²²⁾ which is able to be dissolved in DMSO. After HeLa cells were incubated with indicated concentrations of genipin (at 45, 90, 135 µg/ml) for 24 h, cell viability significantly reduced from 88.4 to 33.8% (Fig. 1B). The IC₅₀ value of 50% cell survived by genipin was about 90 µg/ml. Genipin shows cytotoxic and antiproliferative effects on

Fig. 1. Effect of Genipin on the Cell Proliferation and Cell Viability of HeLa Cells

(A) After HeLa cells were treated with different concentrations (at 45, 90, 135 µg/ml) of genipin for 24 h and 48 h, the cell number was counted using a hemocytometer, (B) after HeLa cells were treated with different concentrations (at 45, 90, 135 µg/ml) of genipin for 24 h, the cell viability was determined by MTT assay. Values were presented as means±S.D. of three independent experiments. **p*<0.05; significantly different from the control group.

HeLa cells in a dose-dependent manner.

Morphology Changes in Genipin-Treated HeLa Cells

It has been demonstrated that apoptotic cells incline to be shrinkage.¹⁰⁾ The cytotoxic effect of genipin was evaluated after HeLa cells were treated with indicated concentrations of genipin (at 45, 67.5, 90 $\mu\text{g/ml}$) for 24 h. We clearly observed typical morphology of apoptosis such as cell shrinkage (Fig. 2 arrows), membrane blebbing under inverted phase-contrast microscopy, compared to untreated control cells (Fig. 2). The number of shrunk cells increased in a dose-dependent manner with the increasing genipin (Figs. 2A—D). In addition, the medium with genipin appeared to be dark blue, and the colors were far darker with increasing concentrations of genipin.

Determination of Apoptosis by Genipin Assessment of DNA fragmentation was performed to confirm the effect of genipin on HeLa cells.¹⁰⁾ HeLa cells were incubated with indicated concentrations of genipin (at 45, 90, 135 $\mu\text{g/ml}$) for 24 h. A characteristic ladder pattern of DNA was shown in the genipin-treated HeLa cells by agarose gel electrophoresis (Fig. 3). Genipin led to the appearance of DNA fragments which were 180 bp integer multiples in size in genipin-treated HeLa cells.

Flow cytometry analysis was carried out to further confirm whether apoptosis was the reason that caused genipin-treated HeLa cells death. Apoptotic cells are assumed to contain more hypodiploid DNA which is presented as sub- G_1 peak in flow cytometry.²³⁾ Treatment with genipin for 24 h resulted in a significant increase in the percentage of apoptosis (Fig. 4D). Here shows some representative examples of the sub- G_1 peak in flow cytometry (Figs. 4A—C). Cells in sub- G_1 peak remarkably increased from 3.7 to 35.1% with the gradual increase of genipin concentrations (Figs. 4A—C). The higher concentration of genipin-treated cells contained more hypodiploid DNA, indicated that genipin significantly induced apoptosis in a dose-dependent way. These findings confirm the conclusion that the induction of cell death in genipin-treated HeLa cells is due to apoptosis. Consequently, we used the concentrations of genipin from 45 to 90 $\mu\text{g/ml}$ for Western blot analysis.

Fig. 2. Morphological Changes in Genipin-Treated HeLa Cells

The cells untreated (A) and treated with genipin at 45 $\mu\text{g/ml}$ (B), 67.5 $\mu\text{g/ml}$ (C), 90 $\mu\text{g/ml}$ (D) for 24 h, were observed under inverted phase-contrast microscopy (arrows indicate cells of shrinkage; magnification, 400 $\times$).

Cell Cycle Distribution in Genipin-Treated HeLa Cells

In order to investigate the effect of genipin on cell cycle distribution, we have examined the cell cycle progression alterations in genipin-treated HeLa cells. The cells were treated with various concentrations of genipin for 24 h and analyzed by flow cytometry. Genipin caused a marked increase from 23.3 to 50.1% at G_1 phase, and this change was accompanied with a concomitant decrease of cell number at S phase from 47.3 to 25.2%, compared with the untreated control cells (Fig. 5). The increase at G_1 phase suggested that cell cycle was arrested at G_1 phase in a dose-dependent manner in genipin-treated HeLa cells.

Effect of Genipin on Apoptosis-Associated Proteins

Fig. 3. Apoptosis Was Evaluated by the Induction of DNA Fragmentation in Genipin-Treated HeLa Cells

HeLa cells were incubated with indicated concentrations of genipin for 24 h, the fragmented DNA was extracted using a DNA Ladder Detection Kit, and analyzed by electrophoresis.

Fig. 4. Effect of the Genipin on Sub- G_1

Flow cytometry analysis of the apoptosis by genipin in HeLa cells. Representative of sub- G_1 peak in HeLa cells incubated with genipin at 0 $\mu\text{g/ml}$ (A), 45 $\mu\text{g/ml}$ (B) and 90 $\mu\text{g/ml}$ (C), x-axis indicates DNA content; y-axis indicates the number of cells counted. Statistic analysis of apoptosis in HeLa cells (D), values were presented as means of percentage $\pm$ S.D. of three independent experiments. Apo indicates the cells with hypodiploid DNA. Percentage of apoptosis changed in dose-dependent manner in genipin-treated HeLa cells.

JNK, Phospho-Jun, p53 and Bax in HeLa Cells We next examined the signaling pathways involved in genipin-induced apoptosis of HeLa cells. We detected a faint phosphorylated JNK band. In the genipin-treated HeLa cells, the activation of JNK was similar to the control at 45 $\mu\text{g/ml}$ and remarkably increased to about 4-fold the corresponding control level at 90 $\mu\text{g/ml}$ (Fig. 6).

To confirm the activation of JNK, the level of phosphorylated c-Jun protein was examined. Two bands were detected using the anti-p-c-Jun antibody (Fig. 6). This was in accordance with the reports that phosphorylated (p-c-Jun) and more highly phosphorylated c-Jun (pp-c-Jun) were observed in Western blot analysis.^{24,25} Treatment of genipin caused phosphorylation of c-Jun and the amount of phospho-Jun remarkably increased about four folds compared to untreated cells at 90 $\mu\text{g/ml}$ (Fig. 6). Phospho-Jun affected genipin-treated HeLa cells in a dose-dependent manner.

Since pro-apoptotic protein p53 plays a crucial role in

apoptosis and cell cycle arrest at G₁ phase,^{26,27} we determined the effect of genipin on the protein level of p53 in HeLa cells. The expression of p53 protein gradually increased and reached a peak at 90 $\mu\text{g/ml}$ during the period of treatment of genipin (Fig. 7). The expression level of p53 protein increased in a dose-dependent manner.

Because bax is one of the down-stream proteins which p53 targets,²⁸ we detected the protein level of bax in genipin-treated HeLa cells. The amount of bax protein significantly increased to about three folds with the treatment of 90 $\mu\text{g/ml}$ genipin (Fig. 8). The level of bax protein increased in a dose-dependent manner by the treatment of genipin in HeLa cells.

DISCUSSION

Although evidences demonstrate that genipin induces apoptosis in human hepatocarcinoma Hep3B cells through a reduced nicotinamide adenine dinucleotide phosphate

Fig. 5. Effects of Genipin on HeLa Cell Cycle Distribution

The percentages of each cell cycle were evaluated by flow cytometry; values were presented as means of percentage $\pm$ S.D. of three independent experiments.

Fig. 6. Effect of Genipin on Activation of p-JNK and p-Jun Protein in HeLa Cells

Western blot analysis of protein levels in the lysates from untreated and genipin-treated HeLa cells. 45 $\mu\text{g/ml}$, 67.5 $\mu\text{g/ml}$ and 90 $\mu\text{g/ml}$ genipin was incubated with HeLa cells for 24 h. β -Actin was used as an internal control to normalize the amounts of proteins loaded in each lane. Expression levels of the p-JNK and p-Jun protein were quantified with Quantity One software; Values were presented as means $\pm$ S.D. of three independent experiments. * $p < 0.05$; significantly different from the control group.

Fig. 7. Effect of Genipin on Activation of p53 Protein in HeLa Cells

The p53 protein increased in dose-dependent way. Expression level of the p53 protein was quantified with Quantity One software; Values were presented as means $\pm$ S.D. of three independent experiments. * $p < 0.05$; significantly different from the control group.

Fig. 8. Effect of Genipin on Activation of Bax Protein in HeLa Cells

The bax protein increased in dose-dependent way. Expression level of the bax protein was quantified with Quantity One software; Values were presented as means $\pm$ S.D. of three independent experiments. * $p < 0.05$; significantly different from the control group.

(NADPH) oxidase-reactive oxygen species (ROS)-JNK-dependent activation of mitochondrial pathway,⁸⁾ its exact mechanism remains to be determined. Additionally, little researches have been focused on the concentration effects of genipin.

In our investigation, we concluded genipin could remarkably induce cytotoxicity in HeLa cells and inhibit the growth of HeLa cells, we observed the feature of shrinkage and occurrence of cell death in genipin-treated HeLa cells; we also observed that the medium incubated with HeLa cells appeared to dark blue after genipin was added into. Genipin is suggested to be a natural and non-toxic cross-linking reagent.²⁹⁾ Genipin is able to react with amino acids to generate a natural blue pigment,³⁰⁾ so the amino acids which are essential for medium and the genipin resulted in the color of blue. For the same pattern, genipin also is the reason that HeLa cells became bluer after treated with genipin.

We demonstrated that genipin triggered apoptosis in HeLa cells, as evidenced by DNA fragmentation (Fig. 3) and sub-G₁ peak of apoptotic markers detection (Fig. 4). Furthermore, the HeLa cells treated with genipin (90 μ g/ml) for 24 h exhibited a dramatic accumulation (50.1%) of cells at G₁ phase of the cell cycle (Fig. 5). Chang *et al.* demonstrated that Penta-acetyl geniposide ((AC)₅GP), another derivative of geniposide, exerted the same effect on cell cycle distribution by induction of phospho-p53 (Ser 392) in rat C6 glioma cells.³¹⁾ It is not fully understood how genipin causes HeLa cells to undergo G₁ arrest and apoptosis. A low level of wild-type p53 protein is presented in non-stressed HeLa cells, however, the level of p53 protein significantly increased in a dose-dependent manner after HeLa cells were incubated with genipin for 24 h (Fig. 7). Recently, p53 has been proposed to induce cell apoptosis by cell cycle arrest at G₁ phase. Oh *et al.* found that p53 arrested cell cycle at G₁ phase by the increasing p53 and p21^{WAF1/CIP1} levels.²⁶⁾ Ganoderic acid Me induced G₁ arrest in wild-type p53 human tumor cells such as lung carcinoma 95-D cells, HCT-116 p53^{+/+} cells.²⁷⁾ Tumor suppressor gene p53 plays a critical role in keeping a balance between cell growth and cell death in normal cell.³²⁾ There was a close correlation between the arrest of G₁ phase and the expression level of p53 protein.³³⁾ This suggests p53 may be able to arrest cell cycle at G₁ phase in genipin-treated HeLa cells.

It was reported that genipin induced the activity of JNK in FaO rat hepatoma cells.⁸⁾ The phosphorylation of JNK was observed in genipin-treated HeLa cell in our study (Fig. 6). The band of phosphorylated JNK was faint after FaO rat hepatoma cells were exposed to genipin for 24 h.⁸⁾ JNK has been reported to participate in lots of apoptotic events. The JNK activation was observed in Daxx-induced apoptosis in HeLa cells³⁴⁾ as well as arsenic trioxide-induced apoptosis in MDA231 cells.³⁵⁾ The effect of JNK was confirmed by the expression of phospho-Jun which increased in a dose-dependent manner after cells were treated by genipin (Fig. 6). These were in accordance with the investigation that the activation of JNK and phospho-Jun associated with apoptosis were also observed in genipin-induced apoptosis in FaO rat hepatoma cells, human hepatocarcinoma Hep3B cells and PC3 human prostate cancer cells.^{8,9)} These results imply that genipin induces apoptotic death in HeLa cells *via* the JNK pathway.

We have stated that p53 plays an important role in cell cycle distribution. In no stressed cells, p53 is in a latent form and the expression level of p53 is pretty low. p53 is suppressed through the binding of murine double minute 2 (Mdm2) and the inactive form of JNK in non-stressed cells.^{36–38)} In response to stress, p53 falls apart from both Mdm2 and JNK, with the phosphorylation of p53.^{36,38)} Phosphorylated p53 resulted in the increase of the p53 protein. p53 has been shown to be phosphorylated *in vivo* by JNK. The activated JNK phosphorylated p53 on Thr-81 in selenite-induced apoptosis of regenerating liver³⁹⁾ as well as monoamine neurotoxins-induced apoptosis in lymphocytes.¹¹⁾ p53 or JNK was suggested to be involved in apoptotic pathway induced by (AC)₅GP in rat C6 glioma cells,^{40,41)} in our study, the induction of both JNK and p53 were observed in genipin-treated HeLa cells. These results suggest that the increase of p53 protein level might be due to stabilization through the phosphorylation, possibly by JNK, which has been activated in genipin-treated HeLa cells.

The accumulation of bax protein could conduce to the induction of mitochondria-dependent apoptosis.²⁸⁾ When the expression of bax protein increases, bax protein translocates from cytosol to the mitochondrial membrane, and leads to the increase of mitochondrial membrane permeability and the release of cytochrome *c*.^{19,42)} Cytochrome *c* can further lead to apoptotic cell death.⁴³⁾ The level of bax protein significantly increased in a dose-dependent manner after HeLa cells were exposed to genipin for 24 h (Fig. 8). The accumulation of bax protein led to apoptotic cell death in genipin-treated HeLa cell. It has been reported that p53 could induce the expression of bax protein.²⁸⁾ Upregulation of p53 and bax protein were observed in Deoxypodophyllotoxin-induced apoptosis in HeLa cells,⁴⁴⁾ and in 6-hydroxydopamine-induced apoptosis in PC12 cells.⁴⁵⁾ The results indicate that the accumulation of bax protein is possibly induced by p53 which has been activated in genipin-treated HeLa cells, and the accumulation of bax protein induces cell apoptotic death eventually.

In conclusion, the data presented in this paper indicate that genipin, metabolite of geniposide, induced HeLa cells apoptotic death and cell cycle arrest at G₁ phase, and genipin affected HeLa cells in a dose-dependent manner. Indeed, our study supports that JNK pathway plays a crucial part in genipin-induced apoptosis, and JNK may contribute to the increased amount of the p53 protein, and bax, which is one of down-stream proteins of p53, induces cell apoptotic death eventually. Further, more study should be carried out to make sure the exact role of JNK, p53 and bax and how they contribute to the genipin-induced apoptosis in HeLa cells. Giving that genipin is a natural and non-toxic reagent and its inhibitory effect on cancer cells, it is considerable for genipin to be used in the treatment of cancer.

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