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# Research Article
# Bakuchiol Induces Apoptosis in Human Hepatocellular Carcinoma Cells HepG2 via Enhancing Bcl-2/Bax/Cyc-t/Caspase-3 Signaling Pathway

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## Abstract

**Background and Objective:** While certain natural plant products exhibit clear anti-cancer properties, the inhibitory effect of bakuchiol (BAK), a natural phenolic compound from the Leguminosae family of Rosaceae, on hepatic cancer and its mechanism remains unreported. This study aimed to explore the inhibitory effect of BAK on Hepatocellular Carcinoma Cells (HepG-2 cells) and to preliminarily investigate its potential mechanism. **Materials and Methods:** In brief, HepG-2 cells were exposed to BAK at concentrations ranging from 0 to 20 μM for 48 hrs. The MTT assay was used to assess the cell inhibition, HE and Hoechst 33342 staining were used to identify the morphological changes of cells and nuclei, apoptosis and cell cycle arrest were detected using apoptosis kit, cell cycle kit and flow cytometry and western blotting was employed to analyzed the expression of key proteins in the apoptotic pathway Bcl-2/Bax/Cyt-c/Caspase-3. **Results:** The BAK significantly inhibited the proliferation of HepG-2 cells. The BAK treatment induced pronounced cellular morphological abnormalities, with enlarged cell gaps, wrinkled cell membranes, shrunken nuclei with deep staining-typical apoptotic features. The BAK-treated group showed marked S-phase arrest, with a significant decrease in the proportion of cells in the G0/G1 phase ($p<0.01$). These changes pronounced with increasing BAK concentration. In the BAK groups, the expression of pro-apoptotic proteins Bax, Cyt-c, Caspase-3, Caspase-9 and p53 was significantly up-regulated and the expression of apoptosis inhibiting protein Bcl-2 was significantly down-regulated. **Conclusion:** The BAK effectively suppressed HepG-2 cells proliferation, likely by inducing apoptosis through enhancing the apoptotic pathway Bcl-2/Bax/Cyt-c/Caspase-3 and by causing S-phase arrest. This study provides an initial insight into the anti-hepatic cancer activity and potential mechanism of BAK, serving as a foundational basis for further in-depth exploration of BAK's anti-cancer mechanisms and its potential clinical applications.

Key words: HepG-2 cells, bakuchiol, apoptosis, Bcl-2/Bax/Cyc-t/Caspase-3 signaling pathway

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Primary hepatic cancer (PHC) is a prevalent global malignancy known for its rapid growth, high aggressiveness, propensity for metastasis and bleak prognosis[1]. Currently, hepatic cancer has become the sixth most common cancer worldwide, with global statistics indicating approximately 780,000 new cases and 740,000 annual fatalities, posing a severe threat to public health[2].

Currently, surgery, radiotherapy and chemotherapy are still the conventional approaches for hepatic cancer treatment, among which clinical surgical resection is the best way, but the recurrence rate after surgery is always high, presenting an enduring challenge in hepatic cancer surgical management. Moreover, surgery does not apply to all hepatic cancer patients and for the majority of those in mid-stage and late-stage, chemotherapy and radiotherapy are the most frequently employed treatments[3,4]. Nonetheless, these two therapeutic approaches often entail severe side effects including gastrointestinal reactions, hair loss, liver and kidney toxicity, neurological toxicity, hypersensitivity reactions and in some cases, cardiotoxicity and bone marrow suppression. In addition, novel techniques like local ablation therapy (radiofrequency ablation, microwave ablation, anhydrous ethanol injection, etc.), interventional therapy and targeted drug therapy can also be used for hepatic cancer treatment and although their efficacy is acceptable, their high cost and limited accessibility pose challenges to widespread adoption. Hence, it is crucial to find effective strategies for hepatic cancer inhibition in order to mitigate the harm caused by this disease.

Triggering apoptosis or cell-cycle arrest in tumor cells is an effective strategy to combat malignant tumors[5,6]. Recent studies have shown that some natural products can induce apoptosis of malignant tumor cells and exert significant cancer inhibitory effects[7,8]. Furthermore, owing to their minimal toxicity and limited side effects, these natural products have garnered attention as potential cancer treatment drugs[9-11]. Bakuchiol (BAK) is a natural phenolic compounds in annual upright herbaceous plants of the Leguminosae family of Rosaceae[12,13]. The BAK has antibacterial, anti-inflammatory, antioxidant and anti-aging properties and recent studies have indicated that it also has significant anti-cancer effects[14]. Studies have indicated that the anticancer effects of BAK are associated with the induction of apoptosis and cell-cycle arrest[15-18]. The Bcl-2/Bax/Caspase-3 signaling pathway is a common pro-apoptotic pathway, previous research demonstrated that the key proteins in this pathway could be remarkably activated by BAK in human lung adenocarcinoma A549 cell line[17].

While the forementioned studies have demonstrated BAK's ability to inhibit cancer cells by inducing apoptosis and cell cycle arrest, its inhibitory effect on hepatic cancer and the underlying mechanism have not been reported in detail. Therefore, the present study aimed to investigate the inhibitory effect of BAK on HepG-2 cells, a commonly utilized cell line for exploring the inhibitory properties of drugs on hepatocellular carcinoma tissues *in vitro*.

## MATERIALS AND METHODS

**Study area:** The experiments were carried out from September 2018 to December 2019 in the Pharmacology Laboratory, College of Medicine, Yanbian University, Yanji, Jilin Province, China.

**Materials and reagents:** The BAK ($\geq$98%) was purchased from Chengdu Ruifenshi Biotechnology Co., Ltd. It was dissolved in DMSO (50 mM) and stored at 4°C. The hepatocellular carcinoma HepG-2 cells were provided by the Department of Pharmacology, School of Medicine, Yanbian University. The HepG-2 cells were cultured in DMEM medium with 10% fetal bovine serum, in a 37°C incubator (Sheldon Manufacturing Inc, Cornelius, Indiana, USA) with and 5% $CO_2$.

**MTT colorimetric test:** About $1\times10^4$ cells were added into 96-well plate and incubated at 37°C, 5% $CO_2$ incubator (Sheldon Manufacturing Inc., Cornelius, Indiana, USA). Once the cells attached to the plate, the DMEM medium was replaced with fresh medium containing different concentration (1.25, 2.5, 5, 10, 20 μmol/L) of BAK. Each group consisted of 6 replicate wells and the control cell was incubated with DMEM with 1 μL DMSO. Wells without cells but containing only DMEM served as the blank control group. Then, after the cells were cultured for 48 hrs, 20 μL MTT (5 mg/mL) was added to each well and incubated for another 4 hrs. After the supernatant was removed, 150 μL DMSO was added and the cells were gently shaken for 10 min to completely dissolve the blue-purple crystals. The absorbance value at 492 nm (OD value) was determined with an automated enzyme labeler (BMG Labtech Inc., Offenburg, Germany) by zero adjustment with a blank control well. The growth inhibition rate of HepG-2 cells by BAK was calculated using the following formula:

$$\text{Inhibition rate}(\%) = \frac{\text{OD control well - ODBAK well}}{\text{OD control well}} \times 100$$

**Hematoxylin and Eosin (H&E) staining:** The HepG-2 cells were cultured on cell crawl in 6-well plate with DMEM medium

containing different concentration (0, 2.5, 5, 10, 20 µmol/L) of BAK for 48 hrs. Then the cells were preserved in 4% paraformaldehyde and stained with Hematoxylin and Eosin (H&E). The morphological changes of cells were observed using a microscope (Chongqing Optoelectronic Instrument Inc., Chongqing, China).

**Hoechst 33342 staining:** The HepG-2 cells were cultured on cell crawl in 6-well plate with DMEM medium containing different concentration (0, 2.5, 5, 10, 20 µmol/L) of BAK for 48 hrs. Then the cells were preserved in 4% paraformaldehyde and stained with Hoechst 33342 staining solution. Then the cell crawls were covered with anti-quenching sealer and the cell morphology was observed using a microscope (Chongqing Optoelectronic Instrument Inc., Chongqing, China).

**Detection of apoptosis by flow cytometry:** The HepG-2 cells were cultured in 60 mm Petri dishes with DMEM medium containing different concentration (0, 2.5, 5, 10, 20 µmol/L) of BAK for 48 hrs. Then the cells were collected and stained according to the instructions of the Annexin V-FITC/PI Apoptosis Kit (Beijing Soleberg Technology Inc., Beijing, China). The cell suspension was filtered through a 200-mesh nylon sieve and the apoptosis of cells were detected by a flow cytometry (Becton, Dickinson and Company, Franklin, New Jersey, USA) within 1 hr and analyzed by FlowJo 10 software.

**Detection of cell cycle by flow cytometry:** The HepG-2 cells were cultured in 60 mm Petri dishes with DMEM medium containing different concentration (0, 2.5, 5, 10, 20 µmol/L) of BAK for 48 hrs. Then the cells were fixed in 70% ethanol at 4°C overnight, then they were further stained with PI staining solution according to the Biotin Cycle Detection Kit (Beyotime Biotechnology Inc., Shanghai, China). The cell suspension was filtered through a 200-mesh nylon sieve and the cell cycle of HepG-2 cells were detected and analyzed through the red fluorescence at 488 nm with the help of Modfit LT software (Becton, Dickinson and Company, Franklin, New Jersey, USA).

**Western blotting:** Protein expression of Cyt-c, Bax/Bcl-2, p53, Caspase-3 and Caspase-9 was assessed through western blot. Briefly, HepG-2 cells were cultured with the treatment of different concentration (0, 2.5, 5, 10, 20 µmol/L) of BAK for 48 hrs. Then the cells were collected and lysed with Ripa lysing solution and PMSF and the total proteins were then obtained with a centrifuge (Sigma-Aldrich Inc., Louis, Missouri, USA) at 12000 g for 20 min at 4°C. The BCA method was utilized to determine the protein sample concentrations and the proteins were then denatured at 100°C for 5 min and stored at -20°C. The protein samples were then separated by 5% stacking SDA-PAGE gel and 10% separated SDA-PAGE gel in an electrophoresis apparatus (Bio-Rad Inc., Hercules, California, USA), transferred onto PVDF membranes with a transmembrane apparatus (Bio-Rad Inc., Hercules, California, USA) and blocked with 5% skim milk for 90 min at room temperature. After washing with 1× TBST for 3 times, the PVDF membranes were then incubated with primary antibodies overnight at 4°C. The PVDF membranes were washed with TBST and further incubated with secondary antibodies for 1 hr at room temperature. The ECL luminescent solution was used to visualize the protein brands and the densitometry of target protein bands was analyzed through the Image Lab software from Bio-Rad (Bio-Rad, Hercules, California, USA).

**Statistical analysis:** The SPSS 20.0 software (SPSS Inc., Chicago, Illinois, USA) were used to statistically analyze the difference with one-way ANOVA methods. Data were presented as Mean±Standard Deviation and $p<0.05$ indicates a significant difference between groups. Graphs were drawn using GraphPad Prism 7 software (GraphPad Software Inc., San Diego, California, USA).

## RESULTS

**BAK inhibits the proliferation of hepatocellular carcinoma HepG-2 cells:** Table 1 demonstrated a significant inhibitory effect of BAK on the proliferation of hepatocellular carcinoma

Table 1: Inhibition rate of BAK on HepG-2 cell (n = 3, $\bar{\chi}\pm$SD)

| | 24 hrs | | 48 hrs | | 72 hrs | |
|---|---|---|---|---|---|---|
| Concentration (µM) | A value | Inhibition rate (%) | A value | Inhibition rate (%) | A value | Inhibition rate (%) |
| 0 | 0.240±0.02 | 00.00 | 0.306±0.03 | 00.00 | 0.160±0.01 | 00.00 |
| 2.5 | 0.182±0.01** | 23.95 | 0.212±0.01** | 30.77 | 0.099±0.01** | 38.08 |
| 5 | 0.154±0.01**## | 35.84 | 0.149±0.03**## | 51.15 | 0.067±0.01**## | 58.13 |
| 10 | 0.082±0.02**ΔΔ | 65.96 | 0.065±0.02**ΔΔ | 78.72 | 0.025±0.01**ΔΔ | 84.17 |
| 20 | 0.004±0.01**&& | 98.51 | 0.002±0.01**&& | 99.35 | 0.003±0.01**&& | 98.00 |

Control group compared with 2.5 µM BAK group: **p<0.01, 2.5 µM BAK group compared with 5 µM BAK group: ##p<0.01, 5 µM BAK group compared with 10 µM BAK group: ΔΔp<0.01 and 10 µM BAK group compared with 20 µM BAK group: &&p<0.01

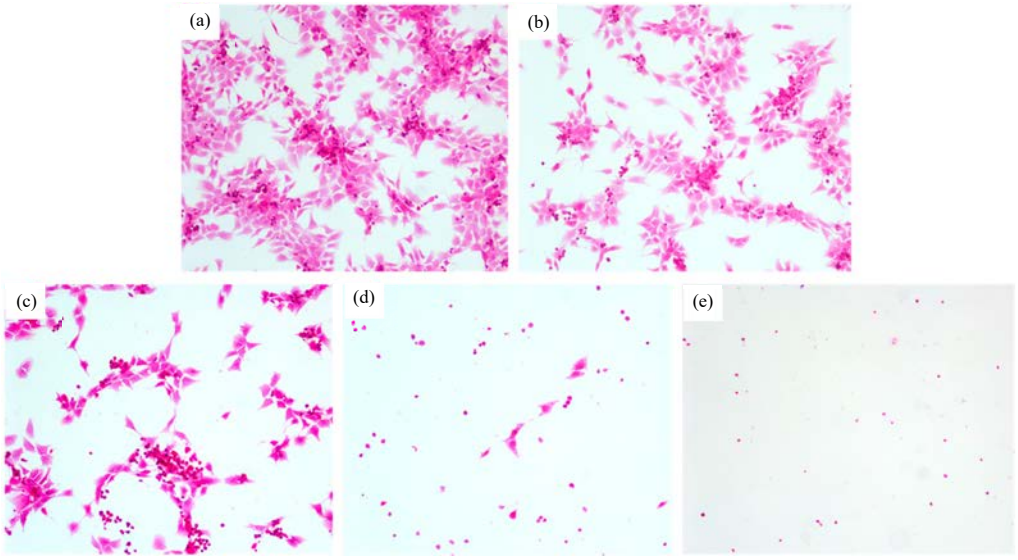


Fig. 1(a-e): Histopathological changes and abnormality of hepatocellular carcinoma HepG-2 cells with BAK treatment, (a) Control group, (b) 2.5 μM BAK group, (c) 5 μM BAK group, (d) 10 μM BAK group and (e) 20 μM BAK group

HepG-2 cells and this inhibitory effect becomes more pronounced with increasing treatment time and drug concentration. When the concentration was 5 μM and the treating time was 48 hrs, the inhibition rate of BAK on HepG-2 cells was 51.15%. This is close to the half inhibition rate, which suggests that the BAK dose of 5 μM at this treatment time can be approximated as the $IC_{50}$ (half maximal inhibitory concentration) of the drug. Therefore, this treatment choses the time and concentration range for further investigation of the BAK's inhibitory effects on HepG-2 cells.

**BAK induced apoptotic morphology in HepG-2 cells:** As shown in Fig. 1, H&E staining revealed that the control group exhibited normal HepG-2 cell morphology with high cell density and uniform staining of both cytoplasm and nucleus (Fig. 1a). While in the BAK treating groups, cell numbers decreased, intercellular spacing increased, cellular debris accumulated, cell nuclei became condensed and deeply stained, displaying distinct apoptotic morphology. These changes became more pronounced with higher BAK concentrations (Fig. 1b-e). When the concentration of BAK$\geq$10 μM, the original morphology of the cells disappeared, the cell membrane ruptured, the cell nucleus was shrunken and the staining was deepened and rounded and the number of cells was obviously reduced, presenting clear signs of cell inhibition and apoptosis morphology (Fig. 1c-e).

To observe nuclear DNA changes more precisely, Hoechst 33342 staining was used in this study. In the control group, cell nuclei had smooth and intact edges, with uniformly light-stained chromatin and no significant reduction in cell numbers was observed (Fig. 2a). Compared with the control group, the cells in the BAK-treated group showed noticeable signs of nuclear consolidation, densely packed nuclear chromatin, intensified fluorescence staining and a decreasing cell count, with larger BAK doses resulting in fewer cells (Fig. 2a-e). In the 20 μM BAK-treated group, cells lost their normal intrinsic morphology, cytoplasm was aggregated, nuclei were condensed and fluorescence staining was enhanced (Fig. 2e). Moreover, cell nucleus fragmentation and apoptotic vesicle formation could be observed, indicating a clear apoptotic cell nucleus morphology and the number of cells was obviously reduced (Fig. 2e).

**BAK induced apoptosis in HepG-2 cells:** Apoptosis detection kit and flow cytometry were used to assess apoptosis of HepG-2 cells. As shown in Fig. 3, compared with the control group, the apoptosis rate was significantly increased after 48 hrs of BAK treatment ($p<0.01$) and the apoptosis rate was higher as BAK's concentration increased (Fig. 3, $p<0.01$).

**BAK induced S-phase cell-cycle arrest in HepG-2 cells:** Cell cycle assay kit and flow cytometry were used to assess the effect of BAK on the cell-cycle of HepG-2 cells. Compared with the control group, the proportion of G0/G1-phase cells was significantly decreased and the proportion of S-phase cells was significantly increased in the BAK groups (Fig. 4; $p<0.01$). Moreover, the proportion of G0/G1-phase cells in the BAK group was significantly decreased and the proportion of

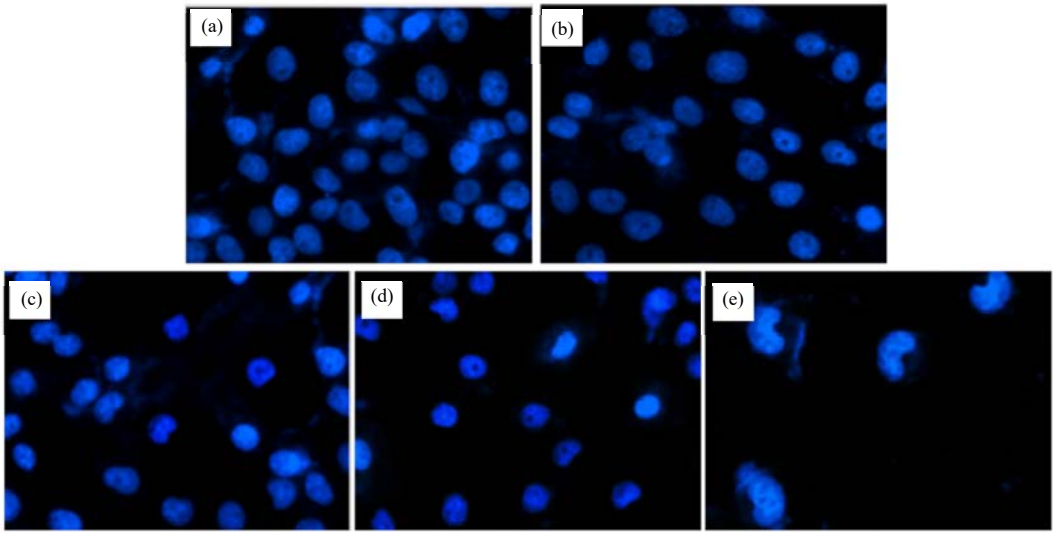


Fig. 2(a-e): Histopathological changes and abnormality of cell nucleus in hepatocellular carcinoma HepG-2 cells with BAK treatment, (a) Control group, (b) 2.5 μM BAK group, (c) 5 μM BAK group, (d) 10 μM BAK group and (e) 20 μM BAK group

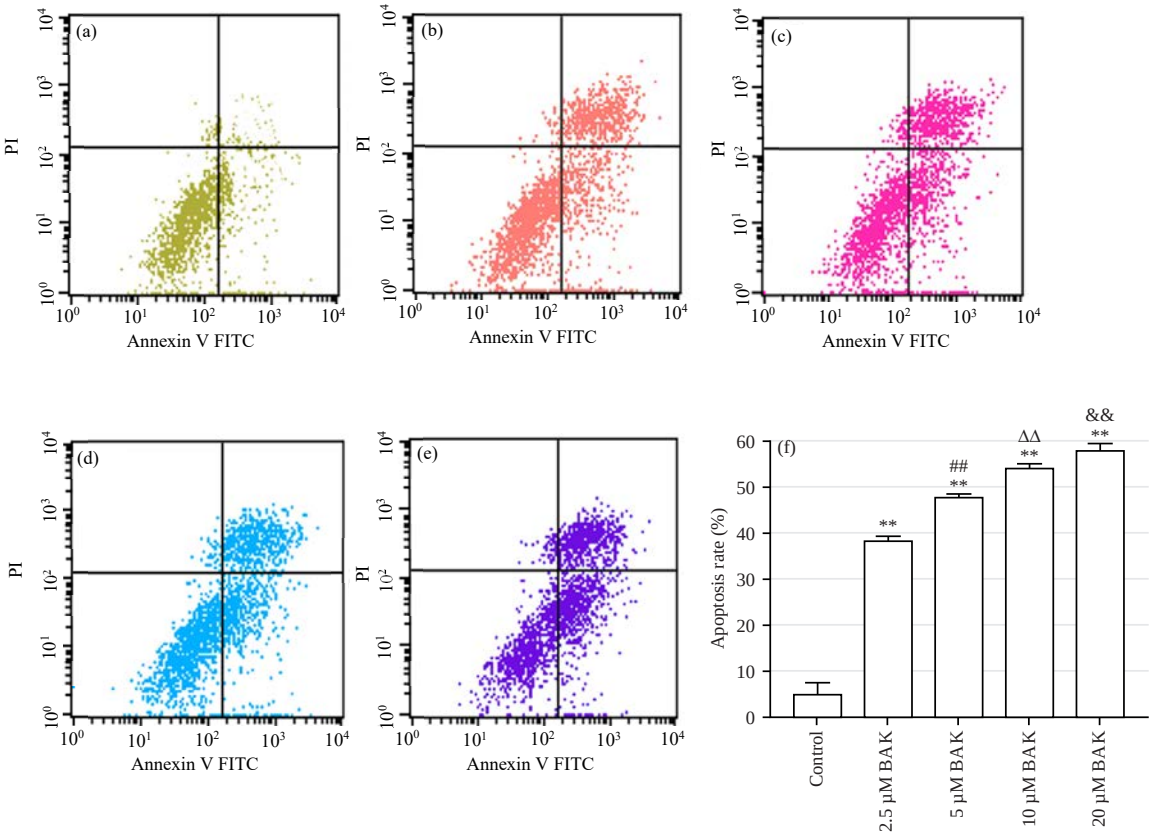


Fig. 3(a-f): Detection of apoptosis rate of HepG-2 cells with BAK treatment by flow cytometry, (a) Control group, (b) 2.5 μM BAK group, (c) 5 μM BAK group, (d) 10 μM BAK group, (e) 20 μM BAK group and (f) Histogram of apoptosis rate of HepG-2 cells

Control group compared with 2.5 μM BAK group: **p<0.01, 2.5 μM BAK group compared with 5 μM BAK group: ##p<0.01, 5 μM BAK group compared with 10 μM BAK group: ΔΔp<0.01 and 10 μM BAK group compared with 20 μM BAK group: &&p<0.01

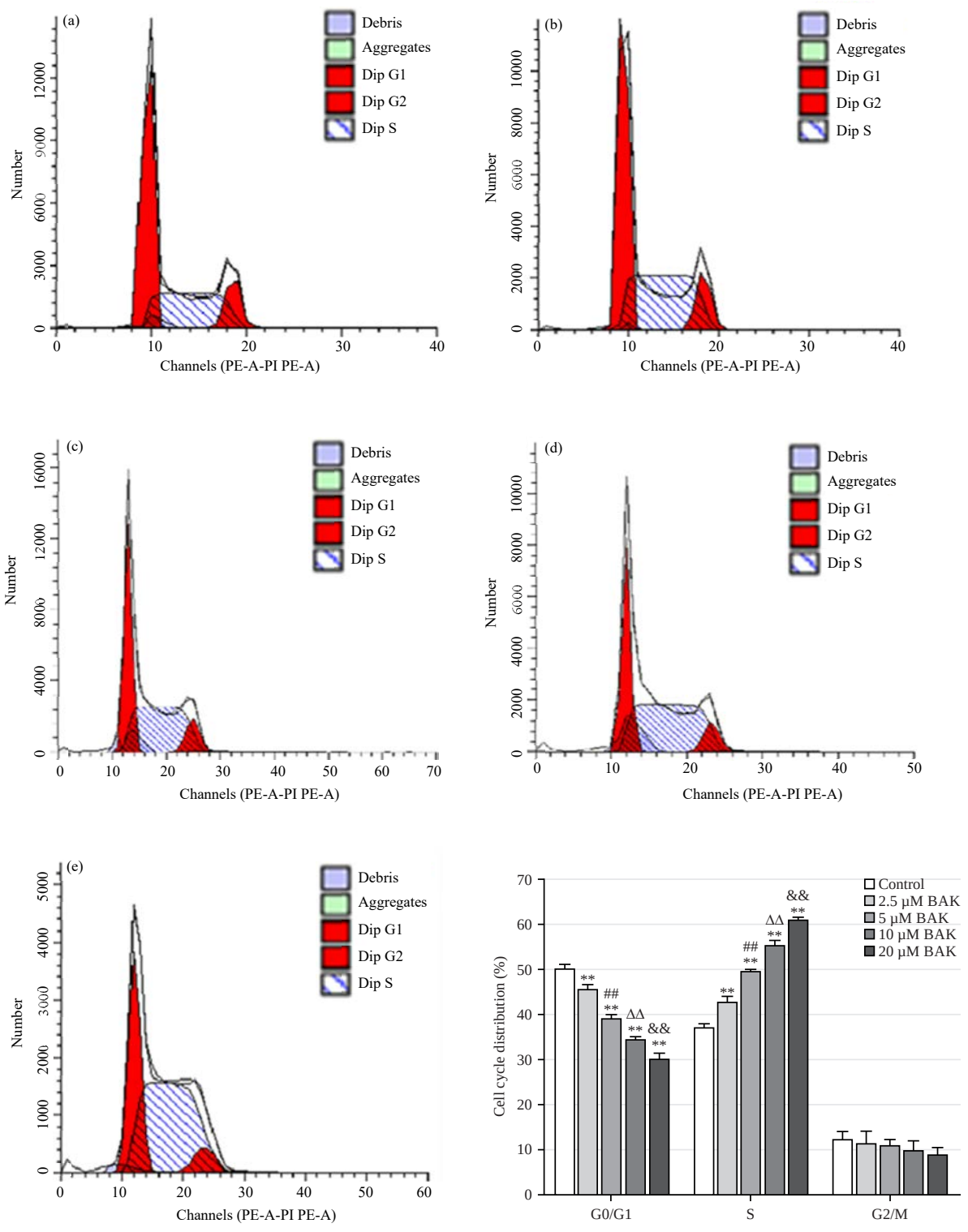


Fig. 4(a-f): Detection of cell-cycle of HepG-2 cells with BAK treatment by flow cytometry, (a) Control group, (b) 2.5 μM BAK group, (c) 5 μM BAK group, (d) 10 μM BAK group, (e) 20 μM BAK group and (f) Percentage of cells in G0/G1, S and G2/M phase

Control group compared with 2.5 μM BAK group: **p<0.01, 2.5 μM BAK group compared with 5 μM BAK group: $^{\#\#}$p<0.01, 5 μM BAK group compared with 10 μM BAK group: $^{\Delta\Delta}$p<0.01 and 10 μM BAK group compared with 20 μM BAK group: $^{\&\&}$p<0.01

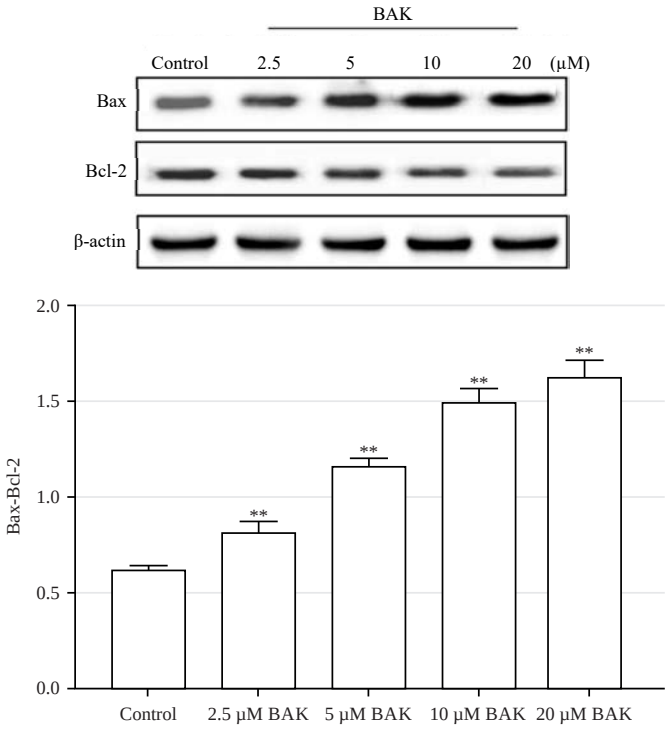


Fig. 5: Levels of Bax and Bcl-2 proteins in HepG-2 cells with BAK treatment
Compared with control group: **p<0.01

S-phase cells was significantly increased with the increase of the BAK concentration, but there was no difference in the proportion of G2/M phase (Fig. 4b-f; p<0.01). These findings suggest that BAK can affect the cell-cycle of HepG-2 cells, leading to a notable S-phase arrest and this arrest effect became more pronounced as the dose of BAK increased.

**BAK enhanced the Bcl-2/Bax/Cyt-c/Caspase-3 apoptosis signaling pathway in HepG-2 cells:** As shown in Fig. 5-8, BAK treatment increased the expression of key proteins in the Bcl-2/Bax/Caspase-3 apoptosis signaling pathway. Figure 5 showed that, compared with the control group, the level of Bax protein was significantly up-regulated in the BAK-treated groups, while Bcl-2 protein was significantly down-regulated and the ratio of Bax/Bcl-2 proteins was gradually increased with the increasing concentration of BAK (Fig. 5; p<0.01). In Fig. 6, it is evident that Cyt-c protein expression significantly increased in the BAK-treated groups when compared to the control group and its level was gradually increased with the increase of the BAK concentration (Fig. 6; p<0.01). Figure 7 and Fig. 8 revealed a significant upregulation in the levels of Caspase-3 and Caspase-9 when HepG-2 cells were treated with BAK (Fig. 7-8; p<0.01).

In addition, the level of P53 protein was also significantly increased in the BAK treated groups (Fig. 9; p<0.01). Taken together, these findings indicated that BAK can enhance the Bcl-2/Bax/Cyt-c/Caspase-3 apoptosis signaling pathway and increase the expression of apoptosis-related protein P53.

## DISCUSSION

Hepatic cancer poses a great threat to human health and triggers such as alcohol consumption and aflatoxin poisoning can promote the occurrence of this type of malignant tumor[19]. Traditional treatments for hepatic cancer, including surgery, radiotherapy and chemotherapy, have inherent limitations, but recently some natural medicines have brought new possibilities for the treatment of hepatic cancer and other malignant tumors[20]. As anticipated in current hypothesis, the results of this study showed that BAK significantly inhibits the proliferation of hepatocellular carcinoma cells HepG-2 cells *in vitro*, induces apoptosis-like morphological changes and significantly increases the overall apoptosis rate of the cells. The BAK has also demonstrated inhibitory effects on various cancer types, including skin cancer[21], breast cancer[15], colon cancer[22], gastric cancer[23], prostate cancer[24] and lung

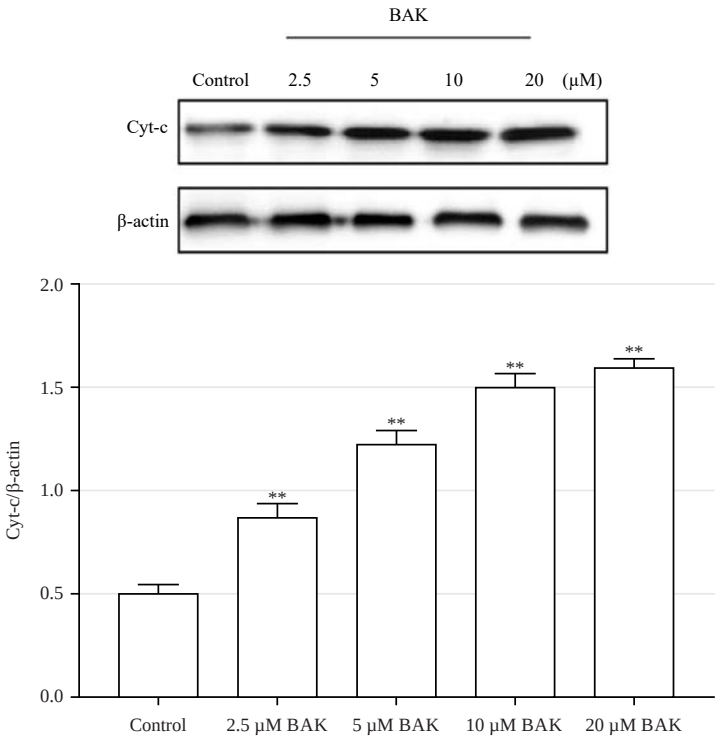


Fig. 6: Levels of Cyt-c protein in HepG-2 cells with BAK treatment
Compared with control group: **p<0.01

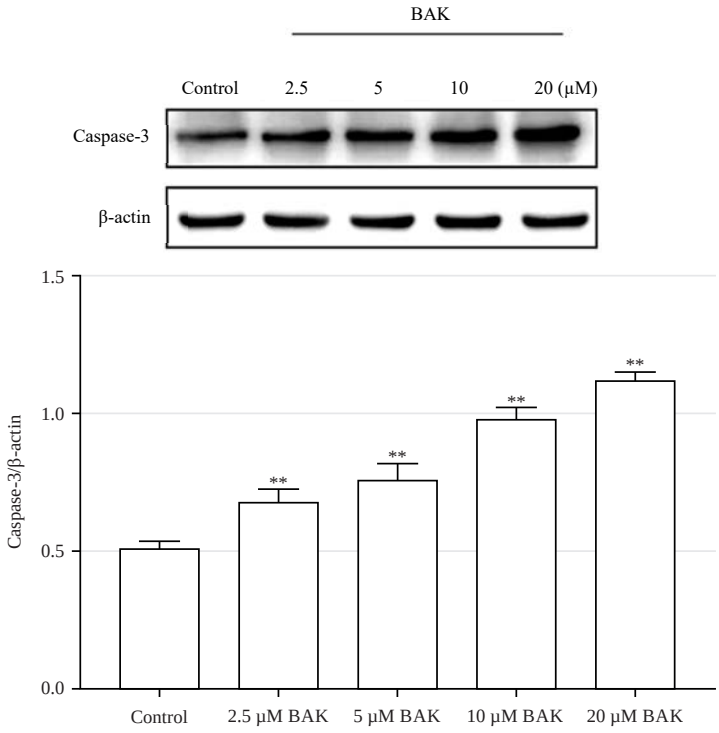


Fig. 7: Levels of Caspase-3 protein in HepG-2 cells with BAK treatment
Compared with control group: **p<0.01

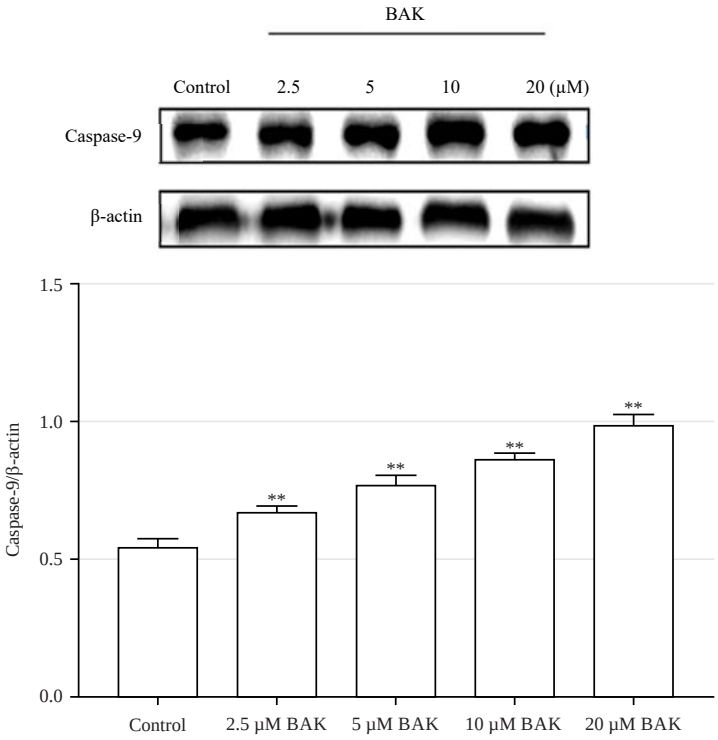


Fig. 8: Levels of Caspase-9 protein in HepG-2 cells with BAK treatment
Compared with control group: **p<0.01

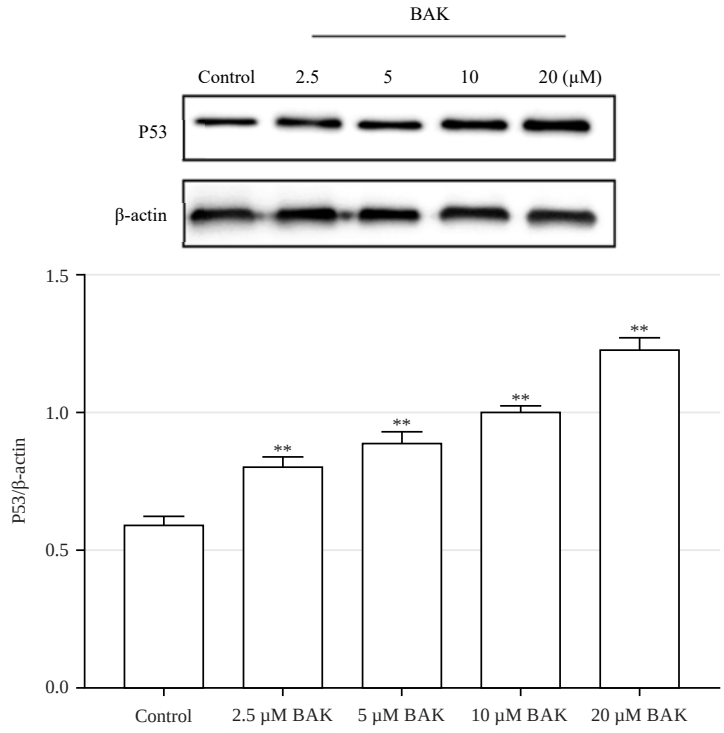


Fig. 9: Levels of P53 protein in HepG-2 cells with BAK treatment
Compared with control group: **p<0.01

cancer[17,25], etc. Together with these studies, the results of the present study reinforce the anti-cancer potential of BAK, offering further support for its potential clinical use.

While BAK has shown good inhibitory effects in these cancer cells, there is a clear difference in the perspectives on its underlying inhibitory mechanism among these studies. In this study, BAK was found to induce remarkable cancer cell apoptosis and abnormal chromatin morphology. A low apoptosis rate is a hallmark of cancer cells, which means the cell proliferation almost unrestricted and the cancerous tissues will expand rapidly *in vivo*. Thus, increasing the apoptosis rate is one of the effective strategies to combat cancer[26]. When treated with BAK for 48 hrs, the normal morphology of the cells was disrupted, cell count significantly decreased, cell nuclei shrank, nuclear chromatin became denser with enhanced fluorescence staining. All these observations indicate the presence of typical apoptotic morphology in hepatic cancer cells. The Hoechst 33342 staining showed that nuclear fragmentation and formation of apoptotic vesicles were observed in HepG-2 cells when treated with BAK.

Protein expression analysis confirmed that the inhibition process was associated with the activation of the Bcl-2/Bax/Cyt-c/Caspase-3 pro-apoptotic pathway. The Bcl-2 and Bax belong to B-cell lymphoma/leukemia 2 apoptosis-related protein family. The Bcl-2 protein is known for its anti-apoptotic properties, while Bax protein promotes apoptosis. When Bax was activated, it was transferred from the cytoplasm to the mitochondria and the process was accompanied by the entry of other small molecules together, which led to the disruption of the mitochondrial membrane and increased permeability. Such changes further facilitate the release of Cyt-c protein into the cytoplasm and triggers the cascade reaction of apoptotic proteins, such as Caspase-3 and Caspase-9, ultimately inducing cancer cell apoptosis[27-29]. Consistent with current finding, Chen *et al.*[17] also found that BAK could increase the levels of Bax, Caspase3/9 and cause more cell apoptosis in lung cancer. But, this does not appear to be the only pathway by which BAK inhibits cancer. It has also been suggested that this apoptosis-inducing effect of BAK on cancer cells is linked to the modulation of phosphoinositide 3 kinase/AKT and mitogen activated protein kinase signaling pathways[23]. The BAK can also regulate the ROS/JNK signaling pathway and promote colon cancer cell apoptosis[22]. The BAK was also discovered to suppress the proliferation of skin cancer cells by directly targeting Hck, Blk and p38 MAP kinase[21]. In this study, we did not delve into the forementioned mechanisms, but we will concentrate on verifying these relevant mechanisms in subsequent studies.

In addition, in line with previous studies, current finding indicated that BAK's anti-cancer activity is linked to its influence on the cell division cycle of cancer cells[15]. Flow cytometry analysis showed that BAK treatment significantly increased the proportion of HepG-2 cells arrested in the S phase. The present study didn't investigate the specific mechanism through which BAK causes S phase arrest, but previous studies have suggested that it may be related to BAK's ability to deactivate the Cdc2 protein[15]. Furthermore, aside from directly inducing apoptosis in cancer cells, BAK can also exert inhibitory effects on cancer through anti-metastasis effects in breast cancer[18]. Hence, the related mechanisms need to be further investigated in detail in future studies. Additionally, BAK has anti-inflammatory[30], antioxidant[31], estrogenic activity[32] and neurotransmitter-regulating activities[33] and whether these activities contribute to its anti-hepatocellular carcinoma process requires further verification.

Although this study revealed the pro-apoptotic effects of BAK in HepG-2 cells and initially found that the process may be associated with enhancing Bcl-2/Bax/Cyt-c/Caspase-3 pro-apoptotic pathway. However, this study did not extensively clarify the dynamic regulation of these proteins in this pathway. In future study, with the help of gene knockdown[34], RT-PCR[35], oncogenic function assay, as well as antagonists of apoptosis pathways, we can understand the specific regulation mechanism underlying the aforementioned pathways more clearly.

## CONCLUSION

Overall, the present study suggested that BAK has a significant inhibitory effect on human hepatocellular carcinoma HepG-2 cells, which may be related to the induction of apoptosis through the Bcl-2/Bax/Cyt-c/Caspase-3 pro-apoptotic pathway and the S-phase arrest of the cells. The present study provides some basis for further investigation of the anti-hepatic cancer activity of BAK and provides a theoretical foundation for the subsequent development of BAK into an anti-tumor natural drug.

## SIGNIFICANCE STATEMENT

The present study aimed to investigate the inhibitory effect of BAK on HepG-2 cells, a commonly utilized cell line for exploring the inhibitory properties of drugs on hepatocellular carcinoma tissues *in vitro*. The findings indicated that the inhibitory effect may be achieved by inducing cell apoptosis through enhancing the Bcl-2/Bax/Cyc-t/Caspase-3

pro-apoptotic pathway, as well as inducing S phase arrest in HepG-2 cells. This study provides an initial insight into the anti-hepatic cancer activity and potential mechanism of BAK, serving as a foundational basis for further in-depth exploration of BAK's anti-cancer mechanisms and its potential clinical applications.

## ACKNOWLEDGMENT

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