

Bakuchiol inhibits cell proliferation and induces apoptosis and cell cycle arrest in SGC-7901 human gastric cancer cells.

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Abstract

The main purpose of the present study was to evaluate the antiproliferative activity of Bakuchiol in human gastric tumor cell lines (SGC-7901) along with demonstrating its mode of action. The effect of this compound on cell viability was evaluated by MTT assay. Fluorescence and phase contrast microscopic techniques were used to study the effect of the compound on cellular morphology and apoptosis. Flow cytometry was used to assess the effect on cell cycle phase distribution. Results revealed that Bakuchiol exerted potent, dose-dependent as well as time-dependent growth inhibitory effects in SGC-7901 cell proliferation with IC50 values of 58.4, 42.3 and 32.5 μM at 12, 24 and 48 h time intervals respectively. On treatment with 10, 50 and 100 μM dose of bakuchiol for 48 h, phase contrast microscope revealed that the cells got detached from one another making clusters of small number of cells floating in the medium. After the cells were treated with 10, 50 and 100 μM concentrations of bakuchiol, cells began to emit orange red fluorescence more heavily at the centre of cells indicating apoptosis. Bakuchiol also induced sub-G1 cell cycle arrest in a dose-dependent manner. The current findings reveal that Bakuchiol is a potent cytotoxic agent against gastric cancer cells and its cytotoxicity is mediated through induction of apoptosis and sub-G1 cell cycle arrest.

Key words:

Gastric cancer, Apoptosis, Bakuchiol, Cytotoxicity, Flow cytometry.

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Introduction

Gastric cancer, which is one of the most common cancers in China, Japan and various East Asian countries, represents the principal cause of death. Despite of the advanced techniques in diagnosis and treatment regimens, this cancer remains a serious health threat throughout the world [1,2]. Gastric cancer treatment involves chemotherapy using cisplatin alone or in combination with other chemotherapeutic agents. Combination chemotherapy with cisplatin as the first-line or second-line treatment for advanced and persistent gastric tumor has yielded decent responses and this treatment is well accepted. One of the important criteria for potential anticancer drugs is the ability to selectively kill tumor, without harming normal cells. But most of the anticancer chemotherapeutic drugs kill normal cells in addition to cancerous cells [3]. This results in the serious, unwanted side-effects of these drugs. So, there is an urgent need to design and develop new anticancer drugs with minimum side-effects and maximum efficacy. Herbal medicines have been reported to be a potential substitute for cancer therapy because of their less toxicity and cheaper prices.

Apoptosis is a programmed cell suicide characterized by chromatin condensation, DNA fragmentation, cell shrinkage and membrane blebbing and apoptotic body formation. The process of apoptosis plays key role in the development of most of the cancers. Most of the tissues that develop cancer indicate a decreased rate of apoptosis process. It has been reported that tumors subjected to radiations and cytotoxic agents showed increased rates of apoptosis, implying that enhanced rate of apoptosis can be used in cancer therapy [4,5]. The objective of the current study was to investigate the anticancer and apoptotic effects of bakuchiol in SGC-7901 human gastric cancer cells along with evaluating its role in inducing cell cycle arrest in these cells.

Materials and Methods

Chemicals and other reagents

Bakuchiol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) were procured from Sigma-Aldrich (St. Louis, MO, USA). Bakuchiol was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) to get

a 100 mM stock solution, which was diluted in the medium to yield the anticipated concentration. An equivalent volume of DMSO in complete culture medium was used as the vehicle control. To exclude the cytotoxicity of DMSO, the ultimate concentration of DMSO for all experiments was kept at less than 0.2%. Minimum essential medium (MEM) and RPMI, fetal bovine serum (FBS), penicillin, streptomycin, trypsin, phosphate-buffered saline (PBS) with calcium chloride and magnesium chloride were obtained from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China). Propidium iodide (PI), acridine orange (AO), Hoechst 33258 were purchased from Boster Biological Technology Co., Ltd. (Wuhan, China).

Cell line and culture conditions

The SGC-7901 human gastric cancer cell line was purchased from the Shanghai Institute of Cell Resource Center of Life Science (Shanghai, China). The cells were cultured in Minimum essential medium (MEM) and RPMI supplemented with 10% (v/v) fetal bovine serum (FBS) under humidified atmosphere of 5% CO₂ at 37°C. The medium was replaced every 3 days. Cells were subcultured every 4 days.

MTT cell viability assay

The SGC-7901 human gastric cancer cells were seeded on a 96-well plate at 2×10^5 cells per well. After 24 h, the cells were treated with bakuchiol at several doses (0, 10, 30, 50, and 100 μM). After incubation times of 12, 24 and 48 h, MTT solution (20 μl) was added. The formazan crystals thus formed were dissolved with DMSO and the absorbance was measured on a microplate reader (FLUOstar Optima, Offenburg, Germany).

Phase contrast microscopy

SGC-7901 human gastric cancer cells were plated in sixwell plates at a density of 2×10^5 cells/ml and then cultured for 24 h. Subsequently, the cells were exposed to treatment with various concentrations of bakuchiol (0, 10, 50 and 100 μM) for 48 h. Following drug treatment, culture plates were examined using an inverted light microscope (Nikon Corp., Tokyo, Japan) and images were captured. DMSO was used as a control.

Fluorescence microscopic assay using acridine orange (AO) and propidium iodide and Hoechst 33258 staining dyes

SGC-7901 human gastric cancer cells were seeded on a chamber slide (Thermo Scientific Nunc Lab Tek II) at cell density of 2×10^5 cells per chamber. The cells were treated with 0, 10, 50 and 100 μM bakuchiol for 48 h. Afterwards, 10 μg/ml of acridine orange (AO) and 10 μg/ml of propidium iodide (PI) were added to each chamber. It was then observed under fluorescence microscope (Olympus IX-70, Tokyo, Japan).

Further, after treating cells with above mentioned doses, the cells were washed with PBS and fixed with 3.5 % formaldehyde for 20 min. After that the cells were again washed removing the fixing solution and then stained with Hoechst 33258. The cells were again washed before analysis under a fluorescence microscope (Olympus IX 81 Tokyo, Japan).

Cell cycle analysis by flow cytometry

The cell cycle analysis was carried out by flow cytometry (Becton-Dickinson FACS Calibur flow cytometry) equipped with CellQuest 3.3 software. After incubation with bakuchiol for 48 h, SGC-7901 human gastric cancer cells were harvested, fixed with 70% ice-cold ethanol for 24 h, treated with 20 μg/ml RNase A (Sigma-Aldrich, St. Louis, MO, USA), stained with 20 μg/ml propidium iodide (PI), and then analyzed by flow cytometer.

Statistical analysis

The results designate values from three independent experiments with the data expressed as the means ± SD. Differences between the control and treatment groups were examined using the Student's t-test with SPSS 17.0 software. A p-value <0.05 was considered statistically significant.

Results and Discussion

Bakuchiol induces potent cytotoxic effects in SGC-7901 human gastric cancer cells

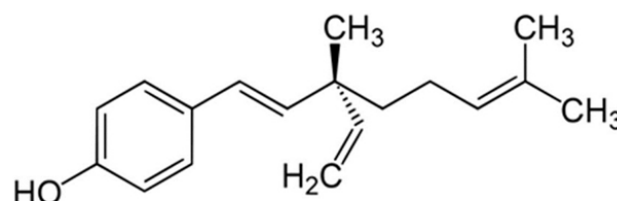


Figure 1: Chemical structure of bakuchiol

The chemical structure and the cytotoxic effects of bakuchiol in SGC-7901 gastric cancer cells are shown in Figure 1 and Figure 2 respectively. As is evident from the figure, bakuchiol induced potent, concentration dependent as well as time-dependent cytotoxic effects in SGC-7901 human gastric cancer cells. The IC₅₀ values of bakuchiol at three different time intervals were found to be 58.4, 42.3 and 32.5 μM respectively at 12, 24 and 48 h time intervals respectively. This indicates that the cytotoxic effect of this compound increases with increase in the incubation time also.

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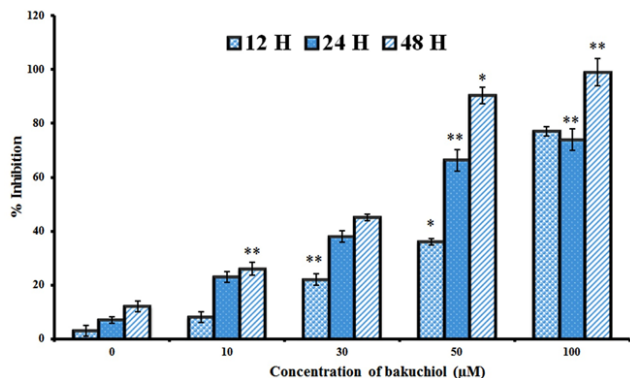


Figure 2: Cytotoxic effect of bakuchiol on the proliferation of SGC-7901 gastric cancer cells. Data are shown as the mean ± SD of three independent experiments. * $P < 0.05$, ** $P < 0.01$, vs 0 μM (control)

Effect of bakuchiol on the cellular morphology of SGC-7901 human gastric cancer cells

In this assay, SGC-7901 gastric cancer cells were exposed to increasing doses of bakuchiol in order to examine any morphological changes induced by this compound. The results of this assay are depicted in Figure 3 A-D indicating that untreated control cells exhibited normal morphology like round shape and were attached to one another. However, on treatment with 10, 50 and 100 μM dose of bakuchiol for 48 h, phase contrast microscope revealed that the cells got detached from one another making clusters of small number of cells floating in the medium. These cells also had uneven shape and incapable to maintain their intact membranes.

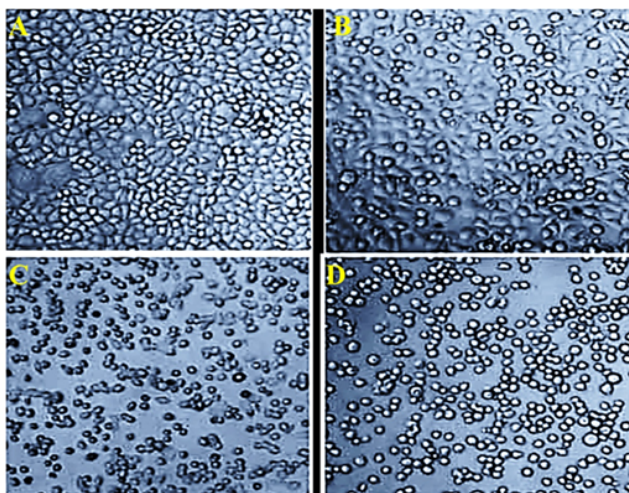


Figure 3: Effects of bakuchiol on the morphology of SGC-7901 gastric cancer cells. Morphological changes were observed under the phase-contrast microscopy after treating without (A, control) and with 10 (B), 50 (C) and 100 μM (D) of bakuchiol for 48 h. Cells got detached from one another making clusters of small number of cells floating in the medium

Bakuchiol induced characteristic morphological features of apoptosis

Fluorescence microscopy using acridine orange and propidium iodide double staining indicated that bakuchiol induced morphological features which are the indications of apoptosis. The results of this assay are shown in Figure 4 A-D revealing that untreated SGC-7901 human gastric cancer cells showed green fluorescence. However, after the cells were treated with 10, 50 and 100 μM concentrations of bakuchiol, these cells began to emit orange red fluorescence more heavily at the centre of cells indicating apoptosis. The number of these apoptotic cells increased with increase in the dosage of bakuchiol.

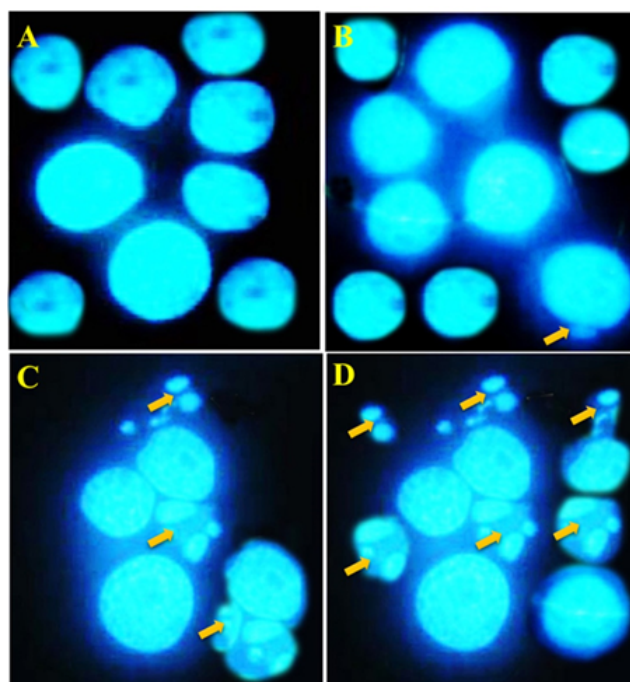


Figure 4: Fluorescence microscopy investigation of SGC-7901 gastric cancer cells using Hoechst 33258 staining dye. The yellow arrows indicate the apoptotic cells which are shrunk and condensed with uneven morphology. The cells were treated without bakuchiol (A, untreated control), and with 10, 50 and 100 μM for 48 h. Results taken from three independent experiments, (Magnification 400 X)

In case of Hoechst 33258 staining, similar results indicating apoptosis were obtained. The results are shown in Figure 5 indicating that unlike control untreated cells which showed normal morphology and spherical shape (Figure 5 A). The bakuchiol-treated cells showed significant chromatin condensation, chromosomal DNA cleavage, blebbing of the membrane and formation of apoptotic bodies. The appearance of these apoptotic bodies was closely related to the dose of bakuchiol (Figure 5 B-D).

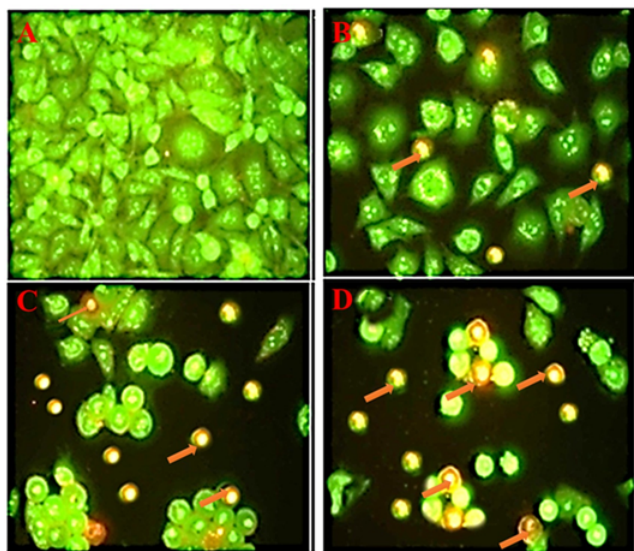


Figure 5: Fluorescence microscopy study of SGC-7901 gastric cancer cells using acridine orange/propidium iodide (AO/PI) staining. The cells were treated without (A, untreated control), and with 10, 50 and 100 μM of bakuchiol for 48 h. The arrows represent apoptotic cells with morphological features including nuclear fragmentation, chromatin condensation and apoptotic body formation

Bakuchiol induced sub-G1 cell cycle arrest in SGC-7901 cells

The fact that bakuchiol induced apoptosis was further confirmed by flow cytometry using propidium iodide as a fluorescent probe. After the SGC-7901 cells were treated with 10, 50 and 100 μM dose of bakuchiol for 48 h, it was observed that there occurred an obvious accumulation of cells in the sub-G1 phase of the cell cycle (also called as the apoptotic phase). The results which are shown in Figure 6 A-D reveal that as compared to the untreated control cells which only showed 1.3% of cells in sub-G1 phase, the cells treated with 10, 50 and 100 μM dose of bakuchiol indicated that the percentage of cells in the sub-G1 phase increased significantly to 6.5%, 23.8% and 62.2% respectively.

Discussion

In the present study, it was observed that bakuchiol induced potent cytotoxic effects in SGC-7901 human gastric cancer cells in a dose and time-dependent manner. Further, using phase contrast and fluorescence microscopic techniques, it was observed that bakuchiol could induce various morphological features which are characteristic of apoptosis including DNA fragmentation, chromatin condensation, blebbing of the membrane and formation of apoptotic bodies. On treatment with 10, 50 and 100 μM dose of bakuchiol for 48 h, phase contrast microscope revealed that the cells got detached from one another making clusters of small number of cells floating in the medium. The cells when treated with 10, 50 and 100 μM concentrations of bakuchiol, these cells began to emit orange red fluorescence more heavily at the centre of cells indicating apoptosis. Flow cytometry revealed that bakuchiol also

induced sub-G1 cell cycle arrest. The cells treated with 10, 50 and 100 μM dose of bakuchiol indicated that the percentage of cells in the sub-G1 phase increased significantly to 6.5%, 23.8% and 62.2% respectively.

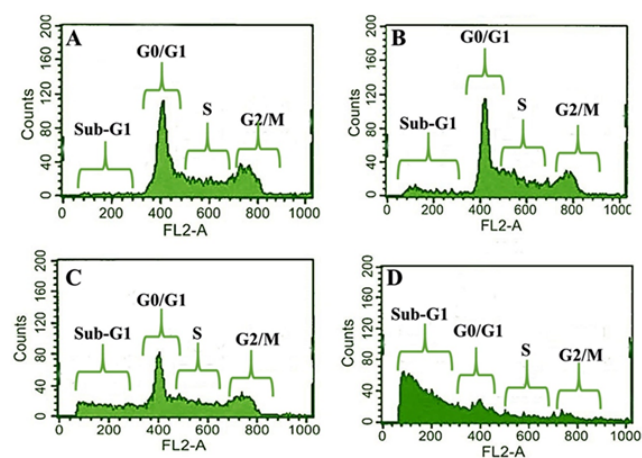


Figure 6: Effect of bakuchiol on the cell cycle phase distribution of SGC-7901 human gastric cancer cells. The cells were treated without (A, untreated control), and with 10, 50 and 100 μM of bakuchiol for 48 h and then analyzed by flow cytometry. Bakuchiol induced sub-G1 cell cycle arrest in these cells leading to an increase in cells in the sub-G1 phase (apoptotic phase)

Natural products have always been used as anticancer agents from many decades. Natural products have played significant role in the design and development of more than 60% of the clinically used anticancer agents. Furthermore, there are numerous natural products or their analogs which are currently in preclinical and clinical stage. World Health organization (WHO) has estimated that about 75 to 80% of the world population rely on traditional medicines for their main health care [6-8]. Natural products which are highly operative and possess less side-effects are a promising substitute for chemotherapy with deadly side-effects. The use of non-cytotoxic bioactive molecules have a great prospective for using against cancer because most of these natural products exhibit pleiotropic properties [9].

Bakuchiol is a terpenoid compound mostly isolated from *Psoralea corylifolia* and *Otholobium pubescens* [10]. Previous studies have reported that bakuchiol exhibits anticancer, hepatoprotective, antihyperglycemic and antibacterial activities [11-13]. To the best of our knowledge, no such study has been published reporting anticancer activity of bakuchiol in SGC-7901 human gastric cancer cells. Therefore, we undertook this objective to study the effect of this compound on this cell line along with studying its mode of action by investigating its effect on cellular apoptosis and cell cycle phase distribution.

Conflict of interest

The authors declare that there is no conflict of interest to reveal with regard to this research work.

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