

Bergapten attenuates human papillary thyroid cancer cell proliferation by triggering apoptosis and the GSK-3 β , P13K and AKT pathways

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Abstract

Background. Over the past few decades, thyroid cancer (TC) incidence has steadily increased globally. The most common TC is human papillary thyroid carcinoma (PTC), which is poorly responsive to the current treatments. Hence, finding a successful therapeutic is urgently required.

Objectives. Bergapten (BG) is a furanocoumarin, a natural psoralen derivative isolated from numerous species of citrus and bergamot oil that has demonstrated anti-tumor activity. However, there are no reports available on the efficacy of BG on PTC cells.

Materials and methods. The current research investigated the anti-cancer activity of BG on human BCPAP cells, with cytotoxicity and apoptosis evaluated using MTT assay, AO/EB, DAPI, PI, ELISA, mRNA, and western blot.

Results. Bergapten (control group, 10 μ M/mL and 15 μ M/mL) inhibited PTC cell proliferation and stimulated apoptosis by enhancing Bax and caspase and reducing Bcl-2, cyclin-D1, c-myc, and survivin in a dose-dependent manner. Furthermore, BG expressively attenuated PI3K/AKT/GSK-3 β signaling, creating an uneven Bax/Bcl-2 ratio that triggered Cyt-c, caspase cascade and apoptosis in human PTC cells.

Conclusions. Our findings emphasize that BG has the potential to be used as a protective natural remedy for human PTC cells.

Key words: apoptosis, thyroid cancer, papillary thyroid cancer, bergapten, PI3K/AKT/GSK-3 β

Cite as

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Background

Thyroid cancer (TC) is the most common endocrine cancer, responsible for 96% of all new and 66.8% of deaths due to endocrine cancers.^{1,2} Papillary thyroid cancer (PTC) is the primary cause behind the rise in TC cases in the USA,³ with PTC subtypes accounting for 74–80% of all TC cases.⁴ The overall death rate from PTC is greater than that of other endocrine cancers, despite recent data on the 5-year survival of TC in China showing notable advancement. Well-differentiated TC is treated employing various methods, including radioactive iodine,⁵ thyroid hormone suppression therapy and surgical excision. However, as TC disparity decreases, standard surgical techniques, including Iodine-131 (I131) excision, no longer produce favorable partially segregated thyroid results.⁶ Hence, novel treatments or potential biological mechanisms for cell cycle regulation, proliferation and apoptosis need to be identified to reduce the prevalence of tumors and improve prognosis in PTC patients.

Natural products were once believed to be crucial for developing novel and effective anti-cancer treatments. The naturally occurring psoralen analog bergapten (BG), a furanocoumarin, is found in the roots and fruits of many different plants, including *Angelica archangelica* L., and it has been demonstrated that BG (5-methoxypsoralen) has anti-proliferative effects against many carcinomas.^{7,8} According to previous research, psoralen can combat the growth of skin and breast cancer cells, and BG prevents the growth of MCF-7-TR1 and MCF-7 cells that are resistant to tamoxifen.⁹ Other research revealed that BG has anti-inflammatory, anti-cancer and hepatoprotective properties,¹⁰ while multiple investigations have shown BG to suppress topoisomerase-1 and COX-2.^{11,12} These results demonstrate that BG stimulates different signaling pathways and possesses promising anti-cancer properties. However, an in-depth understanding of the molecular effects of BG on PTC cells requires more research.

The cell cycle is a highly controlled biological process that includes the G1, S, G2, and M phases in sequence, with its regular progression controlled by several cyclin-dependent kinases (CDKs) and related cyclins. The most critical area of study is cell proliferation, caused by uncontrolled cell proliferation that is central to tumor formation.¹³ Controlled cell death, or apoptosis, is a common target for many management strategies and plays a crucial role in cancer treatment. The morphological changes occurring in dying cells include cell disappearance, membrane blebbing, DNA fragmentation, chromatin reduction, and loss of organelle integrity.¹⁴ Both extrinsic and intrinsic mechanisms may be potential causes of apoptosis,¹⁵ with apoptotic pathways that promote orderly, non-inflammatory cell death connected to caspase cysteine proteases family activation.¹⁶

The PI3K/AKT apoptotic signaling pathway is widely observed in many malignant cells, with numerous

downstream substrates of PI3K/AKT signaling, including GSK-3 β and Bax, contributing to the therapeutic conflict in tumor cells and cell death prevention.¹⁷ According to recent research, human lung carcinoma cells undergoing apoptosis and G1 phase cell cycle arrest were more susceptible to BG than normal lung cancer cells.^{18,19} Therefore, the present study examined BG's ability to inhibit tumor growth in PTC cells and the related signaling cascades.

Objectives

The study aimed to further understand BG functions using BCPAP human PTC cells to assess cytotoxicity and potential molecular effects such as promoting cell viability, cell cycle regulation and apoptosis. Such an approach should provide a preliminary description of BG's ability to inhibit cell proliferation and induce apoptosis in PTC cells.

Materials and methods

Chemicals

Bergapten, RPMI-1640, fetal bovine serum (FBS), antibiotics, phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acridine orange/ethidium bromide (AO/EB), 4',6-diamidino-2-phenylindole (DAPI), propidium iodide (PI), and sodium dodecyl-sulfate (SDS) were obtained from Gibco (Thermo Fisher Scientific, Waltham, USA). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Cayman Chemicals (Ann Arbor, USA), and the antibodies for western blot analysis were acquired from Beyotime Biotechnology (Shanghai, China).

Cell culture

The BCPAP human PTC cells were purchased from Shanghai Aiyuan Biotechnology Co., Ltd (Shanghai, China) and cultured in RPMI 1640 medium containing FBS (10%), penicillin (100 U/mL) and streptomycin (100 U/mL), maintained at 37°C in a humid environment with 5% CO₂.

Cell cytotoxicity assay

The MTT was used to measure cell growth.²⁰ The BCPAP cells were cultured at 37°C in a moist incubator with 5% CO₂ after being seeded into 96-well plates (1 \times 10⁵ cells per well). The cells were separated after overnight incubation, cleaned with PBS, and treated with different concentrations of BG (control group, 2.5, 5, 10, 15, 20, and 25 μ M/mL) for 1 day. To allow the mitochondrial dehydrogenase to transform MTT into insoluble formazan crystals, MTT (100 μ L) solution was added to the control and treated PTC cells, and the cells were incubated for an additional 4 h. The formazan

crystals dissolved when DMSO (150 μ L) was added, and optical density (OD) was determined at 490 nm (Bio-Tek Instruments, Winooski, USA). Using Sigma Plot™ software, 4 parameter logistic function dose-response curves were used to calculate the IC₅₀. Further research was then conducted using the selected concentrations.

Acridine orange/ethidium bromide labelling to assess apoptosis

Acridine orange/ethidium bromide (AO/EB) staining identified apoptotic PTC cell morphology after exposure to BG at concentrations of 10 μ M and 15 μ M.²¹ The BCPAP cells were cultured for a day after adding 10 μ M and 15 μ M BG. The AO/EB dye mixture (100 g/mL of each dye) was added to all groups (control, 10 μ M and 15 μ M BG treated with BCPAP), the unbound dye was removed using a PBS wash, and the cells were left at room temperature for 20 min in the dark before being observed under a fluorescence microscope (IXdrop Standard Microscope; Olympus Corp., Tokyo, Japan).

Examining apoptosis using DAPI staining

Human PTC cells were treated with BG (control, 10 μ M and 15 μ M) in 96-well plates and then fixed with paraformaldehyde (4%) for 10 min at 37°C. The treated BCPAP cells were stained with DAPI for 10 min to analyze the cellular changes associated with apoptosis according to a previously described method.²² The dyed samples were mounted on glass slides and examined using fluorescence microscopy (Nikon Eclipse TS100; Nikon Corp., Tokyo, Japan).

Examining apoptosis using propidium iodide staining

Propidium iodide (PI) staining was employed to examine the apoptotic nuclei of BCPAP cells. The PTC cells were incubated with doses of BG (control, 10 μ M/mL and 15 μ M/mL) for 2 days. Following the incubation, the treated cells were collected and stained with PI.²³ A fluorescence microscope (Nikon Eclipse TS100; Nikon Corp.) was used to observe the red fluorescence emitting from the nuclei.

Measurement of caspase-9 and caspase-8

Apoptosis was determined by measuring caspase-9 and caspase-8 in 3 independent replicates using an ELISA kit following the manufacturer's protocol.

Determination of mRNA expression

Whole RNA was isolated from BCPAP cells according to the company's guidelines using TRIzol® reagent (Abcam, Cambridge, USA). Using a high-capacity complementary DNA (cDNA) reverse transcription kit (Abcam), the extracted

RNA was reverse transcribed into cDNA following the manufacturer's procedures, and the cDNAs were examined using the Fast Start SYBR Green Master mix (Abcam). The band intensity was examined by employing 1.5% agarose gels exposed to electrophoresis and measured using ImageJ v. 1.48 software (National Institutes of Health (NIH), Bethesda, USA):

cyclin D1

F: 5'-CTGTTTGGCGTTTCCCAGAGTCATC-3'

R: 5'-AGCCTCCTCCTCACAACCTCTC-3'

Bcl-2

F: 5'-ATGTGTGTGTGGAGAGGCTCAA-3'

R: 5'-GAGAGACAGCCAGGAAATCAA-3'

Bax

F: 5'-ATGTTTCTGACGGCAACTTC-3'

R: 5'-AGTCCAATGTCCAGCCCAT-3'

caspase-3

F: 5'-TGTTTGTGTGCTTCTGAGCC-3'

R: 5'-CACGCCATGTCATCATCAAC-3'

c-Myc

F: 5'-ACCCTTGCCGCATCCACGAAAC-3'

R: 5'-CGTAGTCGAGGTCATAGTTGGTTGGTGG-3'

survivin

F: 5'-GGACCACCGCATCTACAT-3'

R: 5'-CAAGTCTGGCTCGTTCAGT-3'

Western blot study

Human BCPAP cells were exposed to BG (control, 10 μ M/mL and 15 μ M/mL) and cultured for 1 day. Using an ice-cold lysis solution containing protease inhibitors, cell lysates were formed for western blotting, and the protein content was measured using a Protein BCA Assay Kit (Pierce Chemical Co., Rockford, USA). The proteins were electrophoretically scattered for a brief period and transferred to a polyvinyl fluoride (PVDF) film, which was blocked and then probed overnight at 4°C using 1:1,000 dilutions of P13K, AKT, GSK-3 β , and β -actin primary antibodies. Secondary antibodies (1:5,000) were then added, and the LI-COR Odessey imaging system (Lincoln, USA) was used to stain and visualize the bands to detect the presence of proteins. Densitometry was used to evaluate and quantify the protein band using ImageJ.

Statistical analyses

The statistical analysis employed GraphPad Prism v. 8.0.2 (GraphPad Software, San Diego, USA) and IBM SPSS software v. 25 (IBM Corp., Armonk, USA), with data presented as the median (min and max). Since the sample size was too small to assess data distribution, the differences between groups were analyzed using the non-parametric Kruskal–Wallis test with Dunn's post hoc test. Statistics for the Kruskal–Wallis tests are presented in Table 1 and the Supplementary material. A statistically significant difference was considered when $p < 0.05$. All tests in this study were bilateral.

Table 1. The results of the Kruskal–Wallis test with a correction for controlling I-type error (Dunn's test)

Explained variable	C vs 10 μ M	C vs 15 μ M	10 μ M vs 15 μ M
Caspase-9	0.092	0.01522	0.390
Caspase-8	0.136	0.01522	0.198
Cyclin-D1	0.153	0.01522	0.153
Bcl-2	0.152	0.01522	0.152
Bax	0.154	0.01522	0.154
Caspase-3	0.154	0.01522	0.154
C-Myc	0.154	0.01522	0.154
Survivin	0.153	0.01522	0.153
PI3K	0.152	0.01522	0.152
AKT	0.153	0.01522	0.153
GSK-3 β	0.153	0.01522	0.153

C – control group; 10 μ M – 10 μ M bergapten group;
15 μ M – 15 μ M bergapten group.

Results

Effect of BG on human PTC cells that are cytotoxicity

The MTT assay was used to assess BCPAP human PTC cell cytotoxicity using different doses of BG (control group, 2.5, 5, 10, 15, 20, and 25 μ M/mL). Bergapten improved the cytotoxic and anti-proliferative properties of BCPAP cancer cells in a dose-dependent manner, with a BG dose of less than 10 μ M unable to inhibit PTC cell proliferation. However, when treated with 10 μ M/mL and 15 μ M of BG, 2 PTC cells (BCPAP) showed lower proliferation, and increasing BG to 20 μ M destroyed both PTC cells. The BG IC₅₀ value for both PTC cells was 15 μ M, according to the MTT assay. As a result, the inhibitory concentration levels of 10 μ M/mL and 15 μ M BG were chosen for further testing (Table 2, Fig. 1).

Human PTC cell apoptosis was caused by BG as determined by AO/EB staining

Apoptotic changes were seen in human PTC cells using AO/EB dual-labeling. Untreated BCPAP appeared as uniformly pigmented viable green cells, while PTC cells treated with BG (10 μ M/mL and 15 μ M) exhibited greater apoptotic alterations than the control in a concentration-dependent manner. Human PTC cells treated with 10 μ M BG showed early apoptotic bodies, compacted chromatin

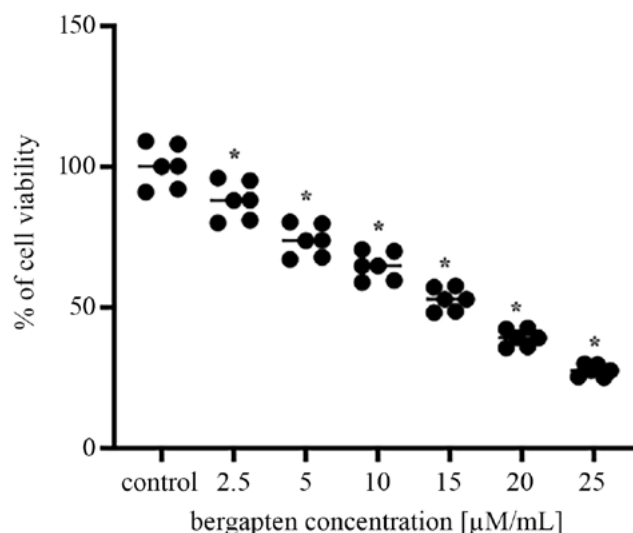


Fig. 1. Bergapten (BG) inhibits human papillary thyroid carcinoma (PTC) cell viability. Human PTC BCPAP cells were treated with various doses of BG (5–20 μ M/mL) for 1 day. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to calculate cell proliferation. This figure presents data (black dots) and the medians (horizontal lines)

* $p < 0.001$ compared to the control group (Table 2)

and vivid greenish-yellow areas caused by membrane blebbing. After 15 μ M BG treatment, BCPAP cells exhibited late apoptotic changes such as chromatin condensation, fragmented nuclei and membrane blebbing. BCPAP cells were orange-red and had lost membrane integrity (Fig. 2).

DAPI staining of human PTC cells

To identify healthy and viable cells, human PTC cells were labeled with DAPI. In contrast to untreated PTC cells, BG-treated BCPAP cancer cells displayed improved nuclear morphology and nuclear body disintegration, leading to apoptosis. When BCPAP cells were exposed to BG (10 μ M/mL and 15 μ M/mL), they experienced chromatin condensation, membrane blebbing, nuclear envelop disintegration, and cellular collapse. These findings showed that BG induced apoptosis in a dose-dependent manner (Fig. 3).

BG triggered apoptosis in human PTC cells, as evidenced by PI staining

PTC cells died in a dose-dependent manner after being treated with BG. Compared to untreated control BCPAP cells, BG (10 μ M/mL and 15 μ M/mL) supplementation

Table 2. Comparison of the studied groups

Variables	Control	2.5 μ M	5 μ M	10 μ M	15 μ M	20 μ M	25 μ M	p-value*
MTT	100.01 (91.00–109.00)	88.02 (80.09–95.93)	73.76 (67.09–80.37)	64.74 (58.90–70.56)	52.91 (48.15–57.67)	39.20 (35.67–42.73)	27.60 (25.12–30.08)	<0.001

10 μ M – 10 μ M bergapten group; 15 μ M – 15 μ M bergapten group. Data were presented as median (min and max); *p-value was generated from Kruskal–Wallis test. There was a significant difference among all groups in the Dunn's test.

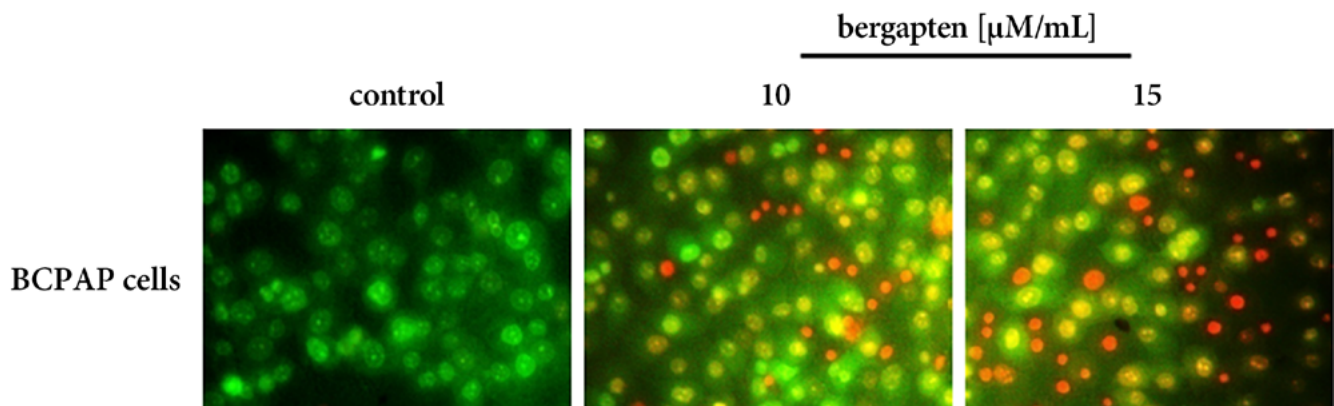


Fig. 2. Assessment of bergapten (BG) on human papillary thyroid carcinoma (PTC) cell apoptosis using acridine orange/ethidium bromide (AO/EB) staining. BCPAP cancer cells were treated with BG (0 μM/mL (n = 6), 10 μM/mL (n = 6) or 15 μM/mL (n = 6)) for 1 day. Human PTC cell apoptosis was studied using AO/EB dual staining and was observed under a fluorescence microscope (Nikon Eclipse TS100; Nikon Corp., Tokyo, Japan)

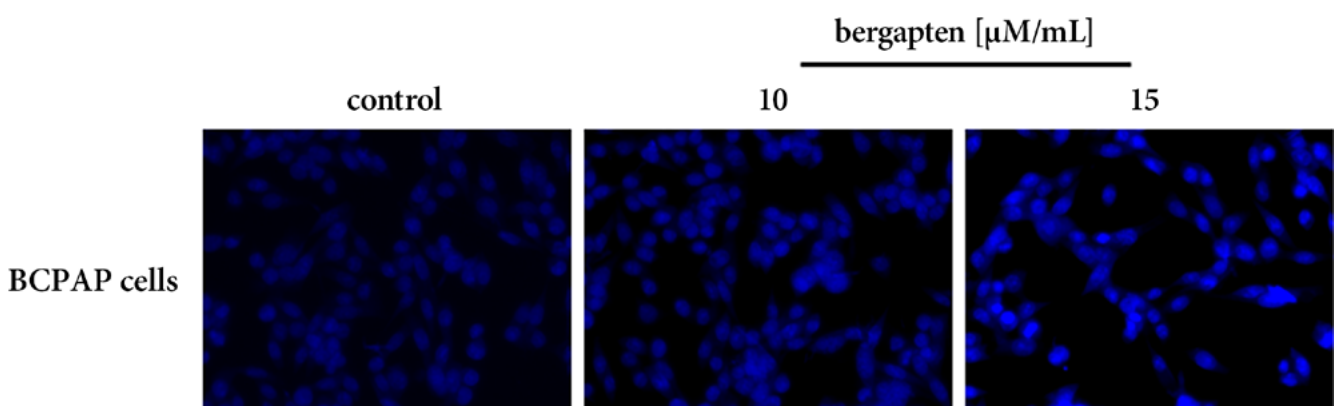


Fig. 3. Evaluation of bergapten (BG) on human papillary thyroid carcinoma (PTC) cells apoptosis using 4',6-diamidino-2-phenylindole (DAPI) staining. The BCPAP cancer cells were treated with BG (0 μM/mL (n = 6), 10 μM/mL (n = 6) or 15 μM/mL (n = 6)) for 1 day. Human PTC cell apoptosis was evaluated using DAPI staining and observed under a fluorescence microscope (Nikon Eclipse TS100; Nikon Corp., Tokyo, Japan)

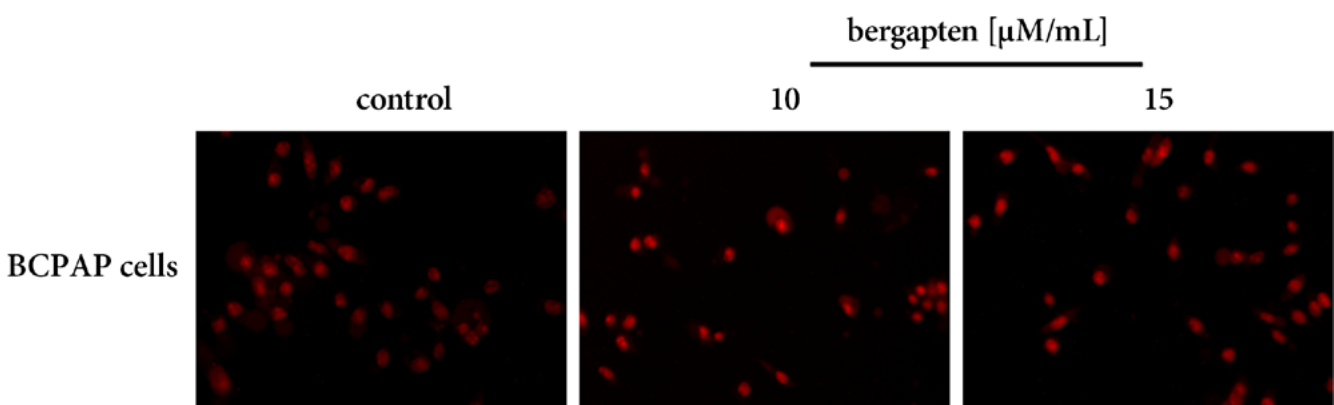


Fig. 4. Effects of bergapten (BG) on human papillary thyroid carcinoma (PTC) cells apoptosis confirmed using propidium iodide (PI) staining. Human PTC cells BCPAP were treated with 0 μM/mL (n = 6), 10 μM/mL (n = 6) or 15 μM/mL (n = 6) BG for 24 h. The BCPAP cancer cell apoptotic activity was assessed with PI staining

boosted apoptotic activity. The apoptotic nuclei of PTC cancer cells were identified using PI labeling. The cells were labeled with PI after the membrane's integrity was compromised, which is related to the loss of membrane polarity and leads to apoptosis. As a result, BG-induced apoptosis inhibited the growth of human PTC cells (Fig. 4).

BG enhanced caspase-8 and caspase-9 levels, as measured using ELISA

Human PTC cells treated with BG had higher caspase-8 and caspase-9 levels than the control. Caspase-8 and caspase-9 levels increased considerably ($p < 0.05$) after 15 μM BG treatment, and the increase was greater than after

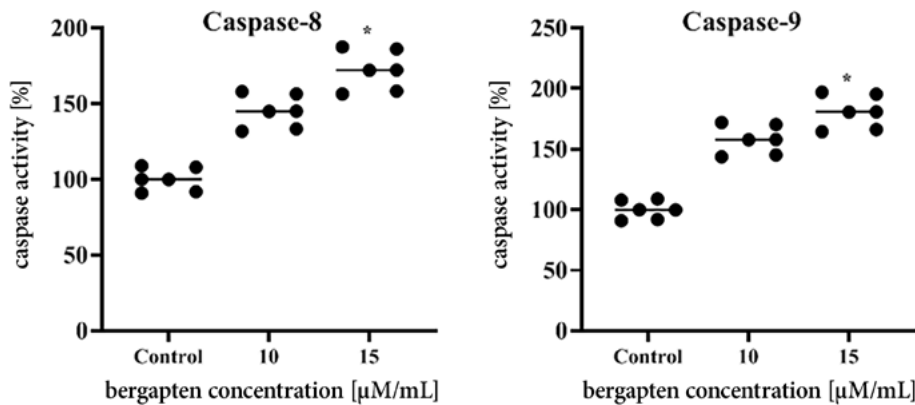


Fig. 5. Measurement of caspase-8 and caspase-9 in bergapten (BG)-treated BCPAP cells. Human papillary thyroid carcinoma (PTC) BCPAP cells were treated with 0 $\mu\text{M/mL}$ ($n = 6$), 10 $\mu\text{M/mL}$ ($n = 6$) or 15 $\mu\text{M/mL}$ ($n = 6$) BG for 24 h. Caspase-8 and caspase-9 levels were measured using enzyme-linked immunosorbent assay (ELISA). This figure presents data (black dots) and the medians (horizontal lines)

* $p < 0.001$ compared to the control group (Table 3).

10 μM BG treatment. Bergapten increased caspase activity in a dose-dependent manner (Table 1,3, Fig. 5).

Effects of BG on BCPAP human PTC cell mRNA expression

BCPAP cells were treated with BG (10 $\mu\text{M/mL}$ and 15 $\mu\text{M/mL}$) to determine mRNA expression due to apoptosis. Cyclin-D1, Bcl-2, c-Myc, and survivin showed higher mRNA levels in untreated BCPAP cancer cells, although Bax and caspase-3 levels were lower. Bergapten significantly lowered the expression of cyclin-D1, Bcl-2, c-Myc, and survivin mRNA while raising Bax and caspase-3 in a concentration-dependent manner (Table 1,3, Fig. 6).

BG suppressed the P13K/AKT/GSK-3 β pathway, as analyzed with western blot

The P13K/AKT/GSK-3 β pathway is involved in the genesis and spread of cancer. Accordingly, P13K, AKT and GSK-3 β protein levels are increased in human PTC cells. P13K/Akt/GSK-3 β levels were lower in both PTC cells after exposure to BG (10 $\mu\text{M/mL}$ and 15 $\mu\text{M/mL}$). In human PTC cells, BG inhibited the expression of these proteins

in a concentration-dependent way. The results showed that BG inhibited PTC cell proliferation and induced caspase-mediated apoptosis by decreasing PI3K/AKT/GSK-3 β signaling (Table 1,3, Fig. 7).

Discussion

Globally, TC primarily affects women, with PTC exhibiting a significant negative impact.^{2,3} There are currently no effective treatments for PTC, though TC can be surgically treated if detected early. However, there are no viable treatments available for those with iodine-resistant or incurable malignancies,^{5,6} which emphasizes the significance of our research on BG's potential anti-cancer benefits to these patients.

The use of compounds produced from natural sources is gaining popularity for elucidating the basic processes of new drugs. According to our findings, BG stimulates a caspase-mediated process in PTC cells that changes multiple cellular pathways and eventually induces apoptosis. This study is the first to establish how BG inhibits PTC cell viability, causes cell cycle arrest, and ultimately triggers apoptosis.

Table 3. Comparison of the studied groups

Variables	Control ($n = 6$)	10 μM ($n = 6$)	15 μM ($n = 6$)	p-value*
Caspase-9	100.05 (91–109)	157.89 (143.61–172.01)	180.84 (164.48–197.02)	0.001
Caspase-8	100.05 (91–109)	145.05 (133.93–158.03)	172.18 (156.61–187.59)	0.001
Cyclin-D1	1 (0.91–1.09)	0.74 (0.67–0.81)	0.56 (0.51–0.61)	<0.001
Bcl-2	1 (0.91–1.09)	0.74 (0.64–0.76)	0.56 (0.45–0.53)	<0.001
Bax	1 (0.91–1.09)	2.10 (1.91–2.29)	3.30 (3–3.60)	<0.001
Caspase-3	1 (0.91–1.09)	1.92 (1.75–2.09)	2.70 (2.46–2.94)	<0.001
C-Myc	1 (0.91–1.09)	0.60 (0.55–0.65)	0.47 (0.43–0.51)	<0.001
Survivin	1 (0.91–1.09)	0.55 (0.50–0.60)	0.39 (0.35–0.43)	<0.001
PI3K	1 (0.91–1.09)	0.73 (0.66–0.80)	0.43 (0.39–0.47)	<0.001
AKT	1 (0.91–1.09)	0.70 (0.64–0.76)	0.41 (0.37–0.45)	<0.001
GSK-3 β	1 (0.91–1.08)	0.80 (0.73–0.86)	0.56 (0.51–0.61)	0.001

10 μM – 10 μM bergapten group; 15 μM – 15 μM bergapten group. Data were presented as median (min and max); *p-value was generated from Kruskal–Wallis test. There was a significant difference among all groups in the Dunn's test.

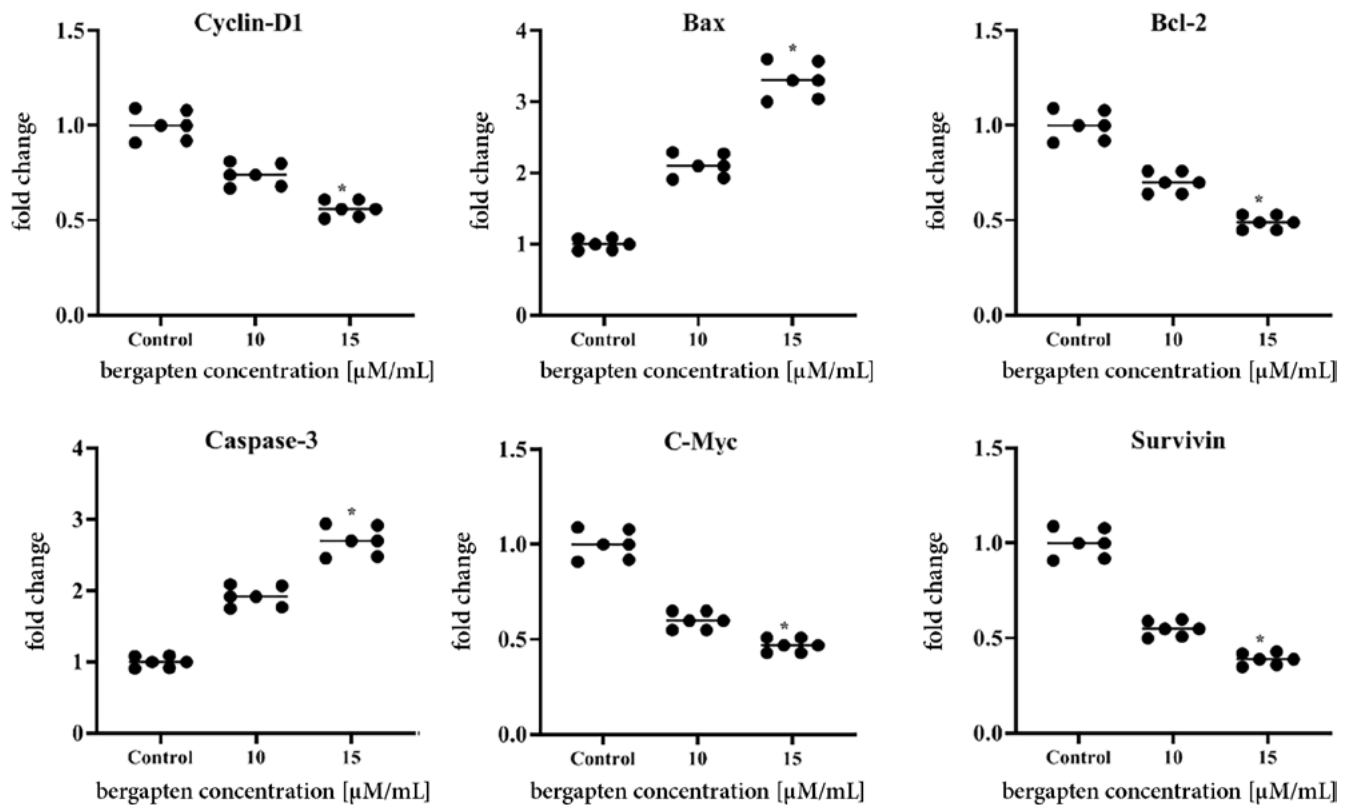


Fig. 6. Influence of bergapten (BG) on the messenger ribonucleic acid (mRNA) expression of human thyroid cancer (TC) BCPAP cells. BCPAP cancer cells were treated with 0 $\mu\text{M/mL}$ ($n = 6$), 10 $\mu\text{M/mL}$ ($n = 6$) or 15 $\mu\text{M/mL}$ ($n = 6$) BG for 24 h. The mRNA levels of cyclin-D1, Bcl-2, Bax, caspase-3, c-Myc, and survivin were determined using reverse transcriptase polymerase chain reaction (RT-PCR) analysis. This figure presents data (black dots) and the medians (horizontal lines)

* $p < 0.001$ compared to the control group (Table 3)

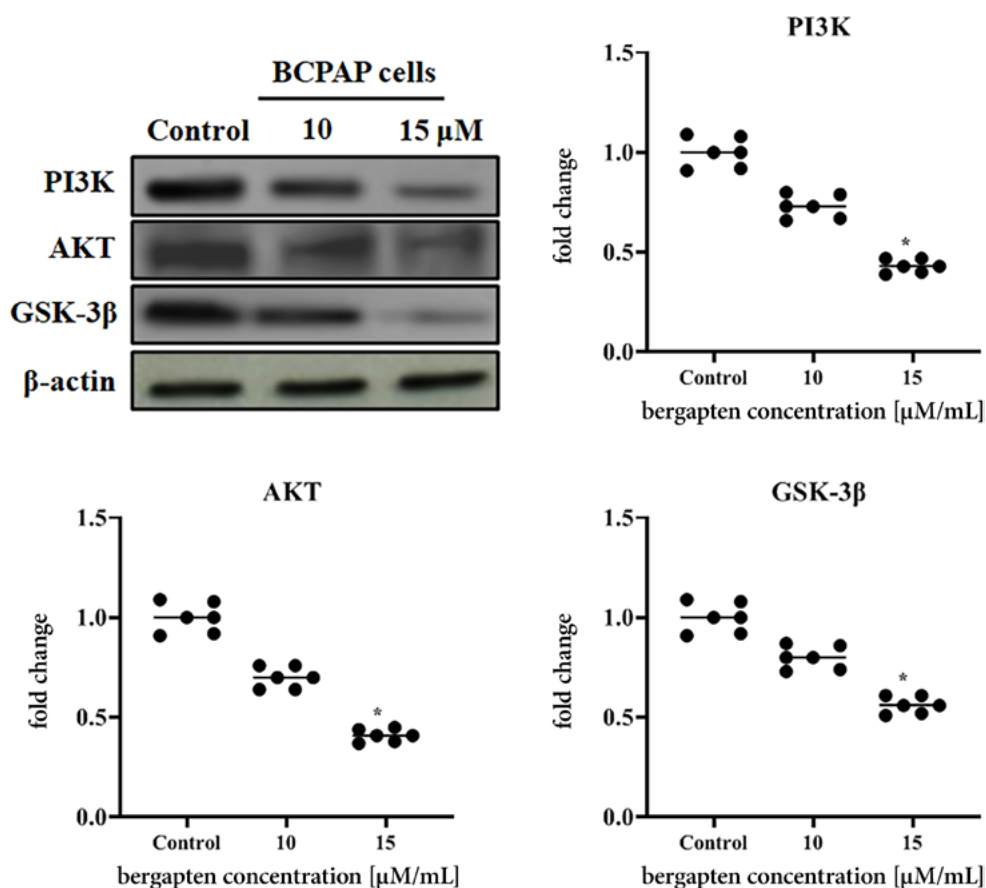


Fig. 7. Effects of BG-treated human papillary thyroid carcinoma (PTC) BCPAP cells on the PI3K/AKT/GSK-3 β pathway. Human PTC BCPAP cells were treated with 0 $\mu\text{M/mL}$ ($n = 6$), 10 $\mu\text{M/mL}$ ($n = 6$) or 15 $\mu\text{M/mL}$ ($n = 6$) BG for 24 h. PI3K, AKT, GSK-3 β , and β -actin protein levels were measured using western blot. This figure presents data (black dots) and the medians (horizontal lines)

* $p < 0.001$ compared to the control group (Table 3)

Our experiments investigated the anti-cancer effects of BG on human PTC cells (BCPAP), and we discovered that BG can decrease the growth of both cells, which may lead to the stimulation of cell cycle arrest and cell death. Since uncontrolled cell growth and proliferation are the fundamental goals of tumor pathogenesis, highlighting the linked unregulated pathways may reduce tumorigenesis.¹³ The research found that BG improved the cytotoxic and anti-proliferative properties of BCPAP cancer cells in a dose-dependent manner, showing that BG has anti-cancer activity by inhibiting PTC cell proliferation when caspase-mediated death and cell-cycle arrest are activated. The cell cycle and apoptosis are 2 critical elements influencing the cytotoxicity rate, with cell cycle checkpoints being an essential component for ensuring high-fidelity cell partition. Apoptosis, a type of cell death, permits other cells to reuse organelles and proteins.¹⁴ We found that BG can successfully stop the cell cycle and trigger apoptosis in PTC cells. Previous research has shown that BG can inhibit the proliferation of certain types of malignant cells, and recent findings suggest that BG can reduce PTC cell viability, including human NSCLC cell lines A549 and NCIH460,^{19,15,24,25} and MEC1,²⁶ as well as hepatocellular carcinoma and bladder cell lines, implying that BG could be used to treat PTC.

Bergapten significantly decreased BCPAP cell growth by triggering apoptotic cell death via the modulation of several apoptosis-related proteins and the production of caspases. Human PTC cells treated with 10 μ M BG showed early apoptotic cells, compacted chromatin and vivid greenish-yellow areas caused by membrane blebbing. After 15 μ M BG treatment, BCPAP cells exhibited late apoptotic changes such as chromatin condensation, fragmented nuclei and membrane blebbing. Furthermore, BCPAP cells were orange-red and had lost membrane integrity. In contrast to untreated PTC cells, BG-treated BCPAP cancer cells displayed improved nuclear morphology and nuclear body disintegration, leading to apoptosis. The apoptotic nuclei of PTC cancer cells were identified using PI labeling after the membrane's integrity was compromised, which is related to the loss of membrane polarity and leads to apoptosis. Using AO/EB, DAPI and PI labeling, the current findings indicate that the anti-tumor effects of BG were achieved through apoptotic induction.

Our findings also revealed that BG reduced the expression of Bcl-2, cyclin-D1, c-Myc, survivin, and Bax. Furthermore, caspase-3, caspase-8 and caspase-9 concentrations were considerably greater in PTC cells. The elevated levels of caspase-8 and caspase-9 revealed that both intrinsic and extrinsic mechanisms were involved in triggering apoptosis.^{15,16} Many anti-cancer medications are thought to induce apoptosis by targeting Bcl-2 family proteins (Bax/Bcl-2), which are critical in determining whether or not cells die. Finally, the Bax protein reduced the potential of the mitochondrial membrane, allowing cytochrome-c to be released and the formation of the homodimer.^{27–29}

According to the gene expression analysis, BG improved cell viability by inducing apoptosis.

Apoptosis may facilitate cell cycle arrest. Cyclins are cell cycle proteins that govern CDK activity, which controls the flipping of cell cycle checkpoint transitions. Ceramide analogs have been shown to trigger apoptosis and G0/G1 cell cycle arrest in PTC cells.³⁰ Similarly, we found that BG-induced cell cycle arrest occurred in BCPAP cells during the G0/G1 phase. Tumor initiation and progression are linked to cell cycle disruption.³¹ As a result, it appears that the cell cycle is one of the primary therapeutic goals for the treatment of neoplasms. The cell cycle's transition from the G1 to the S stage is dependent on one of the cyclins, cyclin-D1, and many carcinomas have been found to have cyclin-D1 hyperactivity.^{32,33} In the course of our investigation, BCPAP cells showed highly expressed cyclin-D1, C-Myc and survivin. Furthermore, BG therapy significantly reduced cyclin-D1, C-Myc and survivin mRNA expression, with previous work showing that BG was capable of restoring normal Bax, caspases, Bcl-2, and cyclin-D1 protein levels in human lung cancer cells A549 and NCIH460.¹⁹ In summary, these findings indicate that BG was cytotoxic, caused BCPAP cell death and restricted their ability to multiply.

The PI3K/AKT/GSK-3 pathway promotes cell survival and apoptosis. AKT is essential for tumor growth and progression by promoting cell proliferation and inhibiting apoptosis.³⁴ AKT phosphorylates GSK-3, which is then involved in the regulation of cell viability, cell cycle progression and anti-apoptotic pathways.³⁵ Bergapten has been recently shown to downregulate the PI3K/Akt/GSK3 pathway in human breast cancer cells.^{7,36} The current study found that BG lowered PI3K, AKT and GSK-3 β phosphorylation, showing that BG affects the PI3K/AKT/GSK-3 β pathway.

Limitations

In this research, we could not perform clinical PTC experiments. Further clinical studies are required on the therapeutic anti-cancer use of BG.

Conclusions

This study evaluated the anti-cancer and apoptotic effects of BG on PTC cells. Our findings revealed that BG inhibits the proliferation of BCPAP human PTC cells and promotes apoptosis via the PI3K/AKT/GSK-3 β pathway by increasing the levels of Bax and caspases while decreasing the levels of Bcl-2, cyclin-D1, c-Myc, and survivin. Our findings show that BG has powerful anti-proliferative and apoptotic properties, making it a promising natural anti-cancer treatment for PTC. In vitro findings demonstrated that BG has anti-tumor effects on PTC cells. However, more research into BG's anti-cancer properties in vivo is needed.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.10602882>. The package contains the following files:

Supplementary Table 1. Results of the Kruskal–Wallis test as presented in Fig. 5.

Supplementary Table 2. Results of the Kruskal–Wallis test as presented in Fig. 6.

Supplementary Table 3. Results of the Kruskal–Wallis test as presented in Fig. 7.

Data availability


The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.


Consent for publication


Not applicable.

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