J Herbmed Pharmacol. 2025; 14(3): 358-365.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2025.52941

Journal of Herbmed Pharmacology

Enhancing intracellular reactive oxygen species, apoptosis, and wound healing activities of Benjakul in cholangiocarcinoma

Sutthiwan Janthamala[1], Saranporn Pornpiphat[1], Anchalee Techasen[2], Malinee Thanee[3], Supaporn Wisungre[4], Sumalin Deechan[4], Nawarat Meechai[4], Phakamas Paratang[4], Kunyarat Duenngai[4*]

[1]Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand
[2]Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand
[3]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand
[4]Department of Thai Traditional Medicine, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, 67000, Thailand

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 5 Feb. 2025
Revised: 3 Apr. 2025
Accepted: 13 Apr. 2025
epublished: 1 Jul. 2025

Keywords:
Thai traditional medicine
Herbal medicine
Antitumor agent
Apoptosis
Cell migration inhibition

ABSTRACT

Introduction: Cholangiocarcinoma (CCA) is an aggressive malignancy of the biliary tract with limited treatment options and a poor prognosis. Current therapies, such as gemcitabine and cisplatin, are associated with notable side effects and limited efficacy. Benjakul (BJK), a traditional Thai herbal formulation, has emerged as a potential anticancer agent. The present study aimed to assess the anticancer effects of BJK on CCA cell lines during cancer progression, focusing on its ability to induce oxidative stress, promote apoptosis and inhibit cell migration.

Methods: BJK extract at concentrations of 50, 100 and 200 µg/mL was applied to CCA, KKU-213B, and KKU-100 cell lines. Intracellular reactive oxygen species (ROS) and apoptosis levels were measured using flow cytometry, whilst cell migration was assessed using a wound-healing assay.

Results: BJK extract increased intracellular ROS levels, particularly in KKU-213B cells, leading to apoptosis. At the concentrations of 50, 100, and 200 µg/mL, BJK significantly induced apoptosis in KKU-213B cells at the rates of 16.2, 51.7 and 56.7% ($P<0.05$), respectively, whilst its effects on KKU-100 cells were minimal. Furthermore, BJK demonstrated potent anti-migratory effects, significantly inhibiting the migration of KKU-213B cells in the wound-healing assay ($P<0.01$).

Conclusion: BJK may exert its anticancer effects through intracellular ROS-mediated apoptosis and migration inhibition, with the combination of its constituent herbs enhancing its therapeutic potential. Furthermore, the results highlight the potential of BJK as a promising therapeutic agent for CCA. By leveraging the anticancer properties of traditional herbal formulations, BJK may improve treatment outcomes for patients with this challenging disease.

Implication for health policy/practice/research/medical education:
This study supports the use of Benjakul as a potential plant extracts treatment for CCA. It enhances understanding of its anticancer effects via intracellular ROS-mediated apoptosis and migration inhibition, informing future research, integrative oncology, and medical education.

Please cite this paper as: Janthamala S, Pornpiphat S, Techasen A, Thanee M, Wisungre S, Deechan S, et al. Enhancing intracellular reactive oxygen species, apoptosis, and wound healing activities of Benjakul in cholangiocarcinoma. J Herbmed Pharmacol. 2025;14(3):358-365. doi: 10.34172/jhp.2025.52941.

***Corresponding author**: Kunyarat Duenngai,
Email: Kunyarat.d@pcru.ac.th

Introduction

Cholangiocarcinoma (CCA), an epithelial cell malignancy that develops along the biliary system, can be categorized into intrahepatic, perihilar, and distal types (1). Infection with liver fluke, *Opisthorchis viverrini* (OV), is a major oncogenic factor associated with fluke-related CCA, and OV infection represents a notable public health concern in Southeast Asia (2). Chronic biliary tract inflammation due to OV infection is the primary risk factor for cholangiocarcinogenesis (3). The symptoms of CCA vary depending on the location of malignancy development, and an accurate diagnosis is difficult to achieve, because CCA is generally asymptomatic in the early stages and difficult to distinguish from other gastrointestinal cancers (4). Currently, the standard treatment for CCA relies on gemcitabine and cisplatin (5), which are associated with considerable side effects, high costs, a limited survival time of patients and toxicity to normal cells (6). Although technological and medical advancements have enhanced the efficacy of chemotherapy, alternative therapies may provide a valuable approach to enhance patient outcomes (7).

Thai traditional medicine, based on natural products, serves an important role in the alternative therapies. In Thai traditional medicine, it is believed that the purpose of drug treatments is to combine medicinal plants with comparable tests in order to increase their therapeutic efficacy. By contrast, plant active components and their pharmacological mechanisms are still insufficiently recognized (8). Previous studies have documented the potential anti-inflammatory, anticancer and antioxidant effects of Thai medicinal plants through *in vitro* assays, and the results indicate that *Gynura pseudochina* var. *hispida* and *Oroxylum indicum* demonstrate potential as anti-inflammatory compounds. Furthermore, *Pouzolzia indica* and *Rhinacanthus nasutus* exhibit anticancer properties by inhibiting the proliferation of leukaemia CCRF-CEM cells and cervical cancer HeLa cells (9). Additionally, Trikatuk, a traditional Thai formulation consisting of three herbs: *Piper nigrum*, *Piper retrofractum,* and *Zingiber officinale*, has demonstrated antioxidant activity with the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) free radicals (10).

Benjakul (BJK), a Thai traditional herbal formulation, is officially included in the Thai National List of Essential Medicines. This formulation comprises five Thai natural herbs: *Piper retrofractum* Vahl., *Piper sarmentosum* Roxb., *Piper interruptum* Opiz., *Plumbago indica* L., and *Zingiber officinale* Rosc (11). Piperine and 6-shogaol, which are the highest components in BJK ethanolic extracts, have exhibited cytotoxic effects on the cell proliferation of breast cancer MCF7 cells (12). BJK has also been reported to suppress the proliferation of human small cell lung cancer NCI-H1688 cells (13). Additionally, 6-shogaol, a compound derived from BJK extracts, has been reported to induce G2/M phase cell cycle arrest and promote apoptosis in human non-small cell lung cancer NCI-H226 cells (14). Furthermore, BJK has shown anti-inflammatory effects by inhibiting the production of prostaglandin E2 (PGE2), a key mediator involved in acute inflammation. The suppression of PGE2 suggests that BJK may serve a role in reducing inflammatory responses (15). In our previous study, BJK exhibited a stronger inhibitory effect on CCA cell proliferation compared with the individual plants (11). Building on these findings, this study aimed to further assess the anticancer mechanisms of BJK, with a particular emphasis on its ability to induce apoptosis and inhibit migration in CCA cells. The results of the present study may offer an alternative approach to cancer treatment and improve the efficacy of inhibiting the progression of cancer cells.

Materials and Methods

Herbal plant collection and identification

The BJK formulation includes five herbal plant species: *Piper retrofractum* Vahl., *Piper sarmentosum* Roxb., *Piper interruptum* Opiz., *Plumbago indica* L., and *Zingiber officinale* Rosc; each of these herbs were gathered from the northern region of Thailand. The verification of the plant materials was performed by comparing them with samples archived at the herbarium of the Southern Center of Thai Medicinal Plants, Faculty of Pharmaceutical Science, Prince of Songkla University (Songkhla, Thailand; herbarium nos. SKP 146161803, SKP 146161907, SKP 146160902, SKP 148160901, and SKP 206261507, respectively).

Reagents and chemicals

The chemicals used in the present study were purchased from Sigma-Aldrich (Merck KGaA). Ham's F-12 Nutrient Mixture and fetal bovine serum were purchased from Thermo Fisher Scientific, Inc.

CCA cell lines

CCA cell lines, KKU-213B (KKU-213A derivative) and KKU-100, were purchased from the Japanese Collection of Research Bioresources (Osaka, Japan). CCA cells were cultured in Ham's F-12 with 10% fetal bovine serum and penicillin/streptomycin (100 U/mL and 100 µg/mL), and then incubated at 37 ˚C in a 5% CO_2 humidified incubator.

Preparation of ethanolic extract

The extraction of BJK was performed as a single batch to ensure a sufficient quantity for use throughout the entire experiment. Fresh medicinal herbs were washed thoroughly, air-dried and subsequently placed in a drying oven at 60 ˚C for 24-72 hours until completely dried. The dried herbs were then finely ground using a milling machine. Each herb was individually weighed to obtain

20 g, and all five herbal components were mixed to yield a total of 100 g of the BJK herbal mixture. The mixed herbs were then macerated in 95% ethanol at a ratio of 1:10 (w/v), using 1000 mL ethanol. The mixture was thoroughly stirred, sealed tightly and left to stand at room temperature for 3 days. After the maceration process, the mixture was stirred again and subsequently filtered through gauze and Whatman No. 1 filter paper. The obtained filtrate was concentrated using a rotary evaporator under reduced pressure in a controlled-temperature water bath. The resulting viscous extract was then subjected to freeze-drying at -96 ˚C until it formed a fine powder, which was subsequently used for the subsequent experimentation. The BJK stock solution (100 mg/mL) was dissolved in 100% DMSO and sonicated for 1 hour. After sonication, the solution was filtered using a 0.2-µm filter and stored at 4 ˚C for further investigation (16).

Intracellular reactive oxygen species (ROS) activity

KKU-213B and KKU-100 cells were seeded in 6-well plates with Ham's F-12 for 24 hours. BJK ethanolic extract at concentrations of 50, 100 and 200 µg/mL, and 0.2% DMSO (maximum percentage of DMSO after dilution from stock) as a control, were added to the plates for 48 hours. Following treatment, the cells were washed with PBS and incubated in Ham's F-12 containing 5 µM CM-H_2DCFDA indicator (Invitrogen™; Thermo Fisher Scientific, Inc.), which was used as an indicator for ROS in the cells for 20 min. After washing with PBS, the cell pellets were collected using trypsinization, and 500 µL propidium iodide (PI) (Invitrogen™; Thermo Fisher Scientific, Inc.) was added. The fluorescence intensity of DCF was then measured using flow cytometry (BD FACS Canto™ II Clinical Flow Cytometry System; BD Biosciences).

Apoptosis assay

The apoptotic cell distribution was assessed using the Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit (cat. no. V13241; Invitrogen™; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. KKU-213B and KKU-100 cells at a density of 200 000 cells/well were seeded in a 6-well plate and incubated for 24 hours. The cells were treated with BJK ethanolic extract at concentrations of 50, 100 and 200 µg/mL, and 0.2% DMSO as a control for 48 hours at 37 ˚C in a 5% CO_2 humidified incubator. After treatment, the cells were trypsinized, washed with cold PBS, and resuspended in 100 µL 1X annexin binding buffer, 2.5 µL annexin V, and 1 µL of 100 µg/mL PI. The stained cells were analyzed using Flow cytometry (BD FACS Canto™ II Clinical Flow Cytometry System; BD Biosciences).

Cell migration assay

Cell migration was assessed using a wound healing assay (17). KKU-213B and KKU-100 cells at a density of 100 000 cells/well were seeded in 24-well plates and incubated at 37 ˚C in a 5% CO_2 humidified incubator until 100% confluence was reached. Scratches were then made using a pipette tip and the cells were subsequently treated with BJK ethanolic extract (50, 100, and 200 µg/mL) or 0.2% DMSO (control). Wound healing was evaluated using an inverted microscope at 24 hours.

Statistical analysis

Data were presented as mean ± standard deviation from two independent experiments. One-way ANOVA followed by Tukey's multiple comparison test was employed to assess differences between groups, with $P<0.05$ considered to indicate a statically significant difference. All statistical analyses and the generation of quantitative bar charts were performed using GraphPad Prism version 8.0 for Windows (GraphPad software, San Diao, CA).

Results

Intracellular ROS activity

The oxidative stress-inducing effects of BJK extract were assessed using flow cytometry by measuring intracellular ROS production. The results revealed that BJK extract induced oxidative stress in KKU-CCA 213B cells, with ROS levels increasing proportional to the extract concentration (Figure 1). Furthermore, BJK extract was demonstrated to slightly induce oxidative stress in CCA cells KKU-100 at the concentration of 200 µg/mL.

Apoptosis analysis

The apoptotic effects of BJK extract were evaluated using the Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit. BJK extract inhibited the growth of CCA cells through the induction of apoptosis. BJK extract significantly induced apoptosis in KKU-213B cells within 48 hours at concentrations of 50, 100 and 200 µg/mL, with apoptotic rates of 16.2 ± 1.273, 51.7 ± 1.414, and 56.7 ± 1.273%, respectively (Figure 2). These results were statistically significant compared with the control group treated with 0.2% DMSO. By contrast, the effect of BJK extract on KKU-100 cells was minimal. BJK extract induced apoptosis in KKU-100 cells at the rates of 4.1 ± 0.141, 5.25 ± 0.636, and 8.55 ± 0.354% at concentrations of 50, 100 and 200 µg/mL, respectively (Figure 3).

Cell migration

To further evaluate the anti-migration properties of BJK, a wound-healing assay was performed to assess its impact on the migration of KKU-213B and KKU-100 cells. The results indicated that BJK extract effectively inhibited the migration of both types of CCA cells, compared with the control group (Figure 4). BJK extract significantly inhibited KKU-213B cell migration at the concentration of 200 µg/mL ($P<0.01$), while it slightly inhibited KKU-100 cell migration at the same concentration.

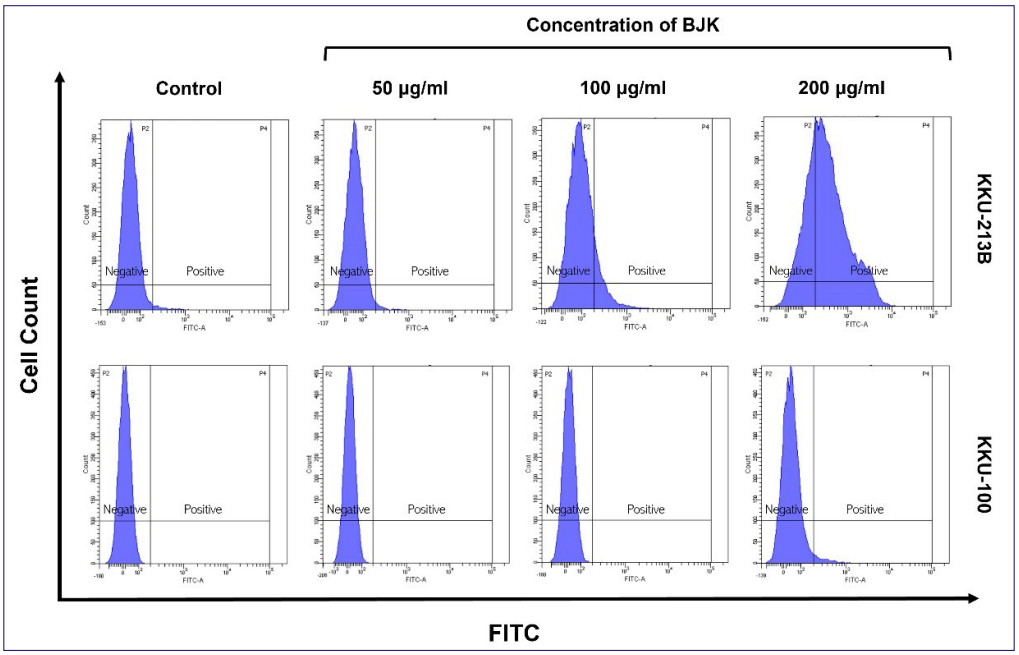

Figure 1. Effect of Benjakul extract on the induction of intracellular ROS on cholangiocarcinoma KKU-213B and KKU-100 cells within 48 h. The x-axis represents fluorescence intensity (FITC), whilst the y-axis indicates cell count. ROS: reactive oxygen species.

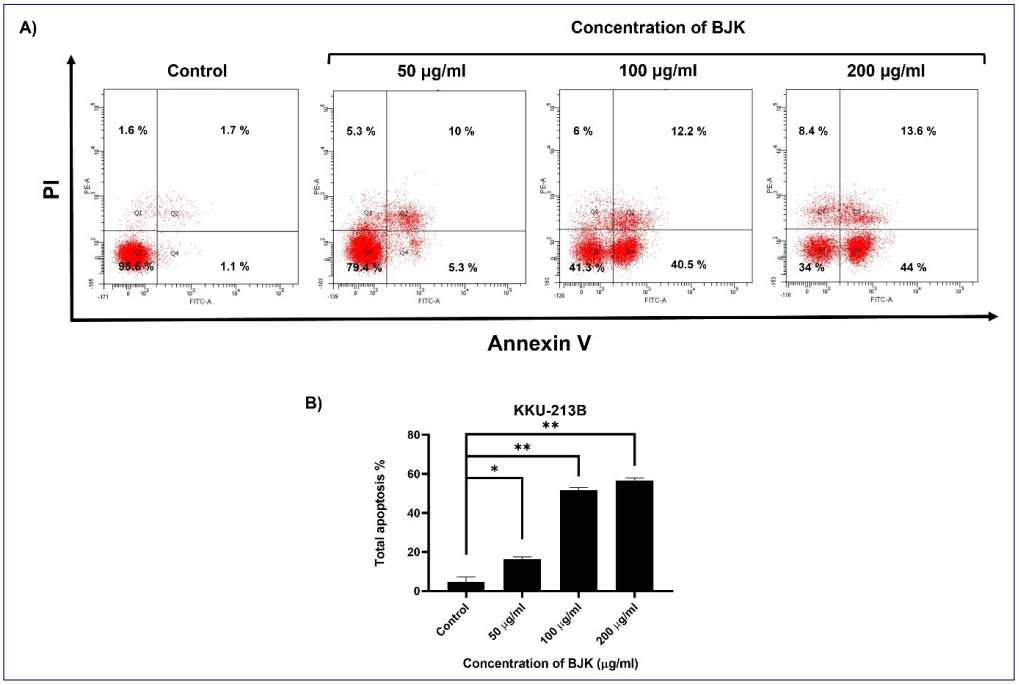

Figure 2. Effect of Benjakul (BJK) extract on the induction of apoptosis in KKU-213B cells after 48 h of treatment. (A) Flow cytometry analysis. (B) Quantitative analysis of apoptosis. $^{*}P < 0.05$, $^{**}P < 0.01$. PI: Propidium iodide.

Discussion

In recent years, there is growing interest in identifying effective herbal treatments and several studies have reported the anticancer potential of herbal extracts against CCA. For example, a previous study reported that ethanol extraction of *Tacca chantrieri* rhizome reduced CCA cell viability by inhibiting growth and inducing apoptosis through an increased Bax/Bcl-2 ratio (18). The extracts from *Oroxylum indicum* have been reported to decrease colony formation in a dose-dependent manner, activate ROS generation and suppress migration in KKU-M452 and KKU-100 cells (19). *In vivo* studies using xenograft

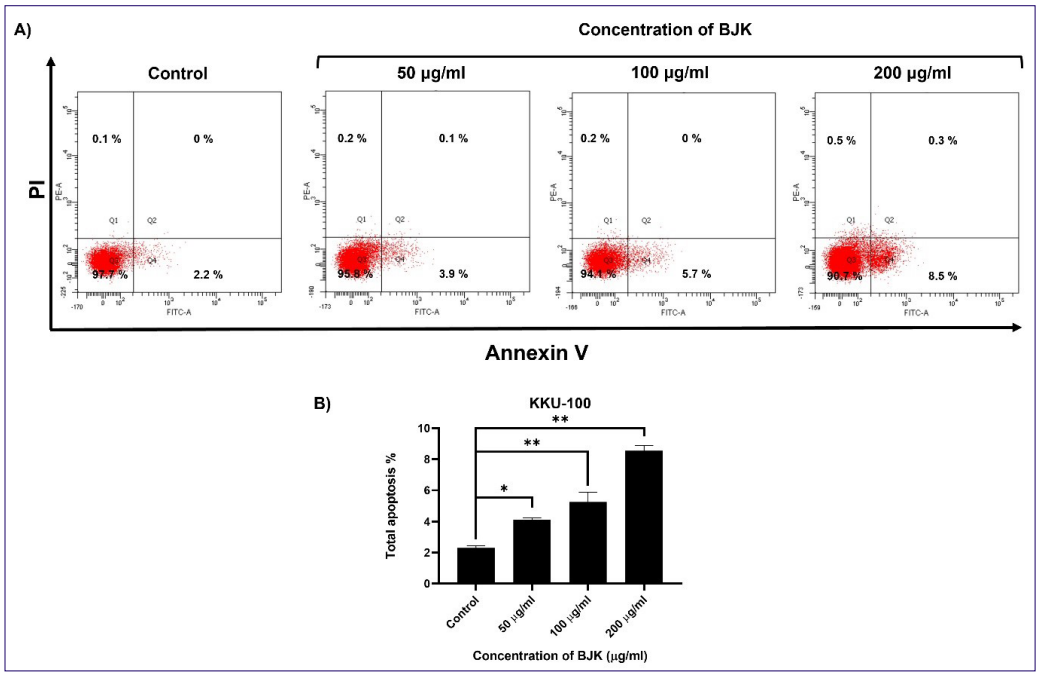

Figure 3. Effect of Benjakul (BJK) extract on the induction of apoptosis in KKU-100 cells after 48 h of treatment. (A) Flow cytometry analysis. (B) Quantitative analysis of apoptosis. *$P < 0.05$, **$P < 0.01$. PI: Propidium iodide.

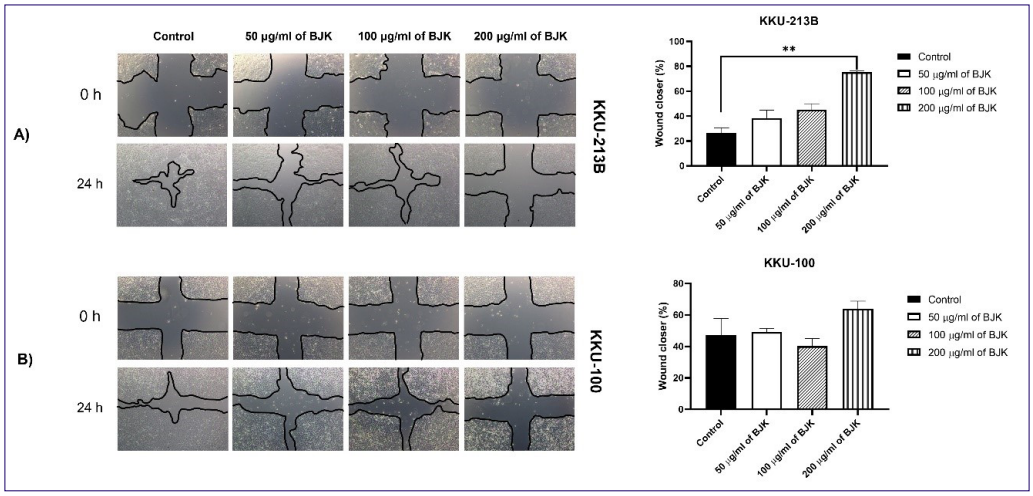

Figure 4. Effect of Benjakul (BJK) extract on the inhibition of cell migration, determined using a wound healing assay on cholangiocarcinoma. **(A)** KKU-213B and **(B)** KKU-100 cells within 24 h of treatment. **$P < 0.01$.

models have also reported the growth-inhibiting and apoptosis-inducing effects of Benja Amarit, a traditional Thai herbal formulation, against CCA. Notably, no significant toxicity to blood cells, kidneys, or liver, and no changes in body weight were reported (20). These findings highlight the potential of natural products in the treatment of CCA and warrant further investigation into their mechanisms of action and therapeutic potential.

The findings of the present study demonstrated the potential of BJK as a therapeutic agent against CCA. The results indicated that BJK extract induced ROS production and promoted apoptosis in CCA cell lines. These findings align with previous studies. In one study, *Piper retrofractum*, one of the herbs included in the BJK, was reported to notably trigger cell death in breast cancer MCF-7 cells, primarily through apoptosis mechanisms. This effect was demonstrated by the increased activity of caspase 3 and enhanced production of ROS (21). In addition, *P. sarmentosum* ethanolic extract was reported to induce a cytotoxic effect and trigger cell death in a human hepatoma cell line (HepG2) through apoptosis (22). Isolated compounds from *P. sarmentosum* were reported to inhibit the growth breast cancer MDA-MB-231 cells by increasing ROS and inducing apoptosis through both

intrinsic (mitochondrial) and extrinsic (death receptor) pathways. They altered the Bcl-2:Bax ratio, promoting mitochondrial dysfunction and caspase activation, leading to cell death (23). Notably, *Plumbago indica* was reported to inhibit the proliferation of lung cancer A549 cells by reducing colony formation and suppressing migration. The anticancer effects occurred through apoptosis induction, ROS generation and mitochondrial dysfunction by increasing active caspase-3 expression and decreasing pro-caspase-3 levels (24).

ROS serves a dual role in cancer biology. Under normal physiological conditions, ROS are involved in cellular signaling and homeostasis. However, an imbalance in ROS levels can contribute to carcinogenesis by inducing DNA damage, promoting mutations and activating oncogenic pathways (25). Conversely, increasing ROS levels beyond the tolerance threshold of cancer cells can trigger oxidative stress-mediated cell death, making ROS induction a promising strategy for cancer treatment (26). In the present study, BJK extract induced intracellular ROS levels beyond the tolerance threshold in CCA cells, leading to apoptosis. This finding aligns with the concept that excessive ROS can overwhelm the antioxidant defenses of cancer cells, resulting in oxidative damage and cell death (27).

The anti-migration effects of BJK observed in the wound-healing assay of the present study are particularly promising, as metastasis is a major challenge in CCA treatment (28). The results revealed that BJK significantly inhibited the migration of KKU-213B cells, with a more pronounced effect compared with that of KKU-100 cells. In addition, this anti-migratory activity is consistent with the properties of the individual herbs in the BJK formulation. For example, *Z. officinale* has been reported to inhibit the migration of breast cancer cells by suppressing matrix metalloproteinases (MMPs) and modulating the epithelial-mesenchymal transition pathway, which are crucial for cancer cell invasion and metastasis (29). Similarly, *P. retrofractum* was reported to markedly inhibit breast cancer MCF-7 cell migration by suppressing MMP-9 levels (21). Additionally, the root extract of *P. indica* has been reported to suppress the migration of cervical cancer HeLa cells in a dose-dependent manner (30). Plumbagin, a naphthoquinone isolated from the root of *P. indica*, has also been reported to have inhibitory effects on the migration and invasion of human CCA cells (CL-6) (31). These findings collectively support the anti-migratory effects of BJK demonstrated in the present study and highlight the potential of its constituent herbs in cancer therapy.

The differential response of KKU-213B and KKU-100 cells to BJK treatment is notable. Whilst BJK significantly induced apoptosis and inhibited migration in KKU-213B cells, its effects were less pronounced in KKU-100 cells. This variability could be attributed to differences in the genetic and molecular profiles of the two cell lines, which could influence their susceptibility to oxidative stress and apoptotic pathways. KKU-100 cells, with a karyotype of 56-92, XXXX, exhibits marked aneuploidy and genomic instability, whilst KKU-213B cells, with a karyotype of 91-109, XX, demonstrates even greater aneuploidy (32). Jamnongsong et al (33) reported that KKU-213B cells harbor mutations in *MAP2K2, TP53, NOTCH2, SMAD4, FLT3,* and *LRP1B* and exhibit heightened ERK pathway activity, making it highly sensitive to pro-growth inhibitors such as MEK, Src-abl, EGFR, and CDK inhibitors. In contrast, KKU-100 cells, with mutations in *SMARCA4, BLM, KEAP1,* and *PTEN*, show lower sensitivity to these inhibitors but demonstrate elevated AKT signaling and greater responsiveness to conventional chemotherapy agents rather than targeted inhibitors. Given the known responsiveness of KKU-213B cells to MEK inhibitors, we hypothesize that BJK exerts its effects through a similar mechanism, leading to its enhanced sensitivity in KKU-213B cells compared with KKU-100 cells. However, the precise molecular interactions between BJK and these pathways remain unexplored. Further investigation into the molecular mechanisms underlying these differences is warranted to optimize the therapeutic potential of BJK for diverse CCA subtypes.

Conclusion

The findings of the present study highlight the potential of BJK as a promising therapeutic agent for CCA. The results demonstrate that BJK extract induces oxidative stress, promotes apoptosis, and inhibits cell migration in CCA cell lines, particularly in KKU-213B cells. These findings underscore the importance of exploring traditional herbal formulations as complementary or alternative therapies for CCA, a disease with limited treatment options and a poor prognosis. By leveraging the therapeutic potential of BJK, it might improve the prognosis and quality of life for patients with this challenging disease. However, further research is needed to fully elucidate the mechanisms underlying the anticancer effects of BJK and to evaluate its safety and efficacy *in vivo*. Future studies should also explore the potential of BJK as part of combination therapies to enhance its therapeutic outcomes.

Acknowledgements

We would like to acknowledge the Institute Research and Development, Phetchabun Rajabhat University and Spandidos Publications English Language Editing Service for English language, grammar, punctuation, spelling and overall style.

Authors' contribution

Conceptualization: Anchalee Techasen, Malinee Thanee, Kunyarat Duenngai.

Data curation: Sutthiwan Janthamala.

Formal analysis: Sutthiwan Janthamala, Kunyarat Duenngai.
Funding acquisition: Kunyarat Duenngai.
Investigation: Sutthiwan Janthamala, Kunyarat Duenngai.
Methodology: Sutthiwan Janthamala, Kunyarat Duenngai.
Resources: Supaporn Wisungre, Sumalin Deechan, Nawarat Meechai, Phakamas Paratang, Kunyarat Duenngai.
Validation: Anchalee Techasen, Malinee Thanee, Kunyarat Duenngai.
Visualization: Saranporn Pornpiphat, Sutthiwan Janthamala.
Writing–original draft: Sutthiwan Janthamala.
Writing–review & editing: Saranporn Pornpiphat, Anchalee Techasen, Malinee Thanee, Supaporn Wisungre, Sumalin Deechan, Nawarat Meechai, Phakamas Paratang, Kunyarat Duenngai.

Conflict of interests

The authors declare no potential conflicts of interest in relation to this study.

Ethical considerations

Regarding human subjects, the research is exempt from ethical considerations.

Funding/Support

This study was supported by the Science Promotion Fund for research and innovation Fiscal Year 2022's innovative research and development plan (65A145000003).

References

1. Brindley PJ, Bachini M, Ilyas SI, Khan SA, Loukas A, Sirica AE, et al. Cholangiocarcinoma. Nat Rev Dis Primers. 2021;7(1):65. doi: 10.1038/s41572-021-00300-2.
2. Sripa B, Brindley PJ, Mulvenna J, Laha T, Smout MJ, Mairiang E, et al. The tumorigenic liver fluke *Opisthorchis viverrini*--multiple pathways to cancer. Trends Parasitol. 2012;28(10):395-407. doi: 10.1016/j.pt.2012.07.006.
3. Titapun A, Luvira V, Srisuk T, Jareanrat A, Thanasukarn V, Thanee M, et al. High levels of serum IgG for *Opisthorchis viverrini* and CD44 expression predict worse prognosis for cholangiocarcinoma patients after curative resection. Int J Gen Med. 2021;14:2191-204. doi: 10.2147/ijgm.S306339.
4. Catanzaro E, Gringeri E, Burra P, Gambato M. Primary sclerosing cholangitis-associated cholangiocarcinoma: from pathogenesis to diagnostic and surveillance strategies. Cancers (Basel). 2023;15(20):4947. doi: 10.3390/cancers15204947.
5. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010;362(14):1273-81. doi: 10.1056/NEJMoa0908721.
6. Yenchitsomanus PT. Cancer immunotherapy: challenges and advancements in CAR T cell technology. Siriraj Med J. 2024;76(5):313-24. doi: 10.33192/smj.v76i5.268031.
7. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer chemotherapy and beyond: current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2023;10(4):1367-401. doi: 10.1016/j.gendis.2022.02.007.
8. Yanda L, Tatsimo SJN, Tamokou JD, Matsuete-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, et al. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022;2022:4205823. doi: 10.1155/2022/4205823.
9. Siriwatanametanon N, Fiebich BL, Efferth T, Prieto JM, Heinrich M. Traditionally used Thai medicinal plants: in vitro anti-inflammatory, anticancer and antioxidant activities. J Ethnopharmacol. 2010;130(2):196-207. doi: 10.1016/j.jep.2010.04.036.
10. Nutmakul T, Chewchinda S. Synergistic effect of Trikatuk, a traditional Thai formulation, on antioxidant and alpha-glucosidase inhibitory activities. Heliyon. 2023;9(1):e13063. doi: 10.1016/j.heliyon.2023.e13063.
11. Duenngai K, Promraksa B, Janthamala S, Thanee M, Sirithawat P, Wisungre S, et al. Antioxidant and anticancer potentials and metabolic profiling of Benjakul, a Thai herbal preparation. Trop J Nat Prod Res. 2024;8(4):6877-83.
12. Sakpakdeejaroen I, Itharat A. Cytotoxic compounds against breast adenocarcinoma cells (MCF-7) from Pikutbenjakul. J Health Res. 2009;23(2):71-6.
13. Rattarom R, Sakpakdeejaroen I, Hansakul P, Itharat A. Cytotoxic activity against small cell lung cancer cell line and chromatographic fingerprinting of six isolated compounds from the ethanolic extract of Benjakul. J Med Assoc Thai. 2014;97 Suppl 8:S70-5.
14. Itharat A, Rattarom R, Hansakul P, Sakpakdeejaroen I, Ooraikul B, Davies NM. The effects of Benjakul extract and its isolated compounds on cell cycle arrest and apoptosis in human non-small cell lung cancer cell line NCI-H226. Res Pharm Sci. 2021;16(2):129-40. doi: 10.4103/1735-5362.310520.
15. Kuropakornpong P, Itharat A, Panthong S, Sireeratawong S, Ooraikul B. In vitro and in vivo anti-inflammatory activities of Benjakul: a potential medicinal product from Thai traditional medicine. Evid Based Complement Alternat Med. 2020;2020:9760948. doi: 10.1155/2020/9760948.
16. Promraksa B, Phetcharaburanin J, Namwat N, Techasen A, Boonsiri P, Loilome W. Evaluation of anticancer potential of Thai medicinal herb extracts against cholangiocarcinoma cell lines. PLoS One. 2019;14(5):e0216721. doi: 10.1371/journal.pone.0216721.
17. Janthamala S, Jusakul A, Kongpetch S, Kimawaha P, Klanrit P, Loilome W, et al. Arctigenin inhibits cholangiocarcinoma progression by regulating cell migration and cell viability via the N-cadherin and apoptosis pathway. Naunyn Schmiedebergs Arch Pharmacol. 2021;394(10):2049-59. doi: 10.1007/s00210-021-02123-0.
18. Armartmuntree N, Kittirat Y, Promraksa B, Loilome W, Dokduang H, Techasen A, et al. Antioxidative and anticancer effects of *Tacca chantrieri* extract enhancing cisplatin sensitivity in cholangiocarcinoma cells. PLoS One. 2025;20(1):e0317111. doi: 10.1371/journal.pone.0317111.
19. Senggunprai L, Prawan A, Kongpetch S, Kukongviriyapan V, Buranrat B. Induction of cell death and apoptosis of *Oroxylum indicum* extract with gemcitabine on human cholangiocarcinoma cells through reducing epidermal growth factor receptor. Pharmacogn Mag. 2022;18(79):729-37. doi: 10.4103/pm.pm_133_22.

20. Yapasert R, Lertprasertsuk N, Subhawa S, Poofery J, Sripanidkulchai B, Banjerdpongchai R. Antitumor efficacy of the herbal recipe Benja Amarit against highly invasive cholangiocarcinoma by inducing apoptosis both in vitro and in vivo. Int J Mol Sci. 2020;21(16):5669. doi: 10.3390/ijms21165669.
21. Buranrat B. Anticancer potential of *Piper retrofractum* on breast cancer MCF-7 cells via induction of cell death and reduction of cell migration. Pharmacogn Mag. 2022;18(79):532-9. doi: 10.4103/pm.pm_455_21.
22. Zainal Ariffin SH, Wan Omar WH, Zainal Ariffin Z, Safian MF, Senafi S, Megat Abdul Wahab R. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. Cancer Cell Int. 2009;9:6. doi: 10.1186/1475-2867-9-6.
23. Hematpoor A, Paydar M, Liew SY, Sivasothy Y, Mohebali N, Looi CY, et al. Phenylpropanoids isolated from *Piper sarmentosum* Roxb. induce apoptosis in breast cancer cells through reactive oxygen species and mitochondrial-dependent pathways. Chem Biol Interact. 2018;279:210-8. doi: 10.1016/j.cbi.2017.11.014.
24. Phiphitphibunsuk W, Buranrat B. *Plumbago indica* L. root extract induces anti-proliferation, anti-migration and apoptosis on the human lung cancer cell line. Indian J Pharm Educ Res. 2024;58(3):861-9. doi: 10.5530/ijper.58.3.94.
25. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24(10):R453-62. doi: 10.1016/j.cub.2014.03.034.
26. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov. 2009;8(7):579-91. doi: 10.1038/nrd2803.
27. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, et al. ROS in cancer therapy: the bright side of the moon. Exp Mol Med. 2020;52(2):192-203. doi: 10.1038/s12276-020-0384-2.
28. Ioffe D, Phull P, Dotan E. Optimal management of patients with advanced or metastatic cholangiocarcinoma: an evidence-based review. Cancer Manag Res. 2021;13:8085-98. doi: 10.2147/cmar.S276104.
29. Lee HS, Seo EY, Kang NE, Kim WK. [6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. J Nutr Biochem. 2008;19(5):313-9. doi: 10.1016/j.jnutbio.2007.05.008.
30. Buranrat B, Wongsuwan P, Phiphitphibunsuk W. Evaluation of anticancer activity of *Plumbago indica* root extract on cervical HeLa cancer cells. Not Bot Horti Agrobot Cluj Napoca. 2023;51(3):12990. doi: 10.15835/nbha51312990.
31. Panrit L, Plengsuriyakarn T, Martviset P, Na-Bangchang K. Inhibitory activities of plumbagin on cell migration and invasion and inducing activity on cholangiocarcinoma cell apoptosis. Asian Pac J Trop Med. 2018;11(7):430-5. doi: 10.4103/1995-7645.237187.
32. Sripa B, Seubwai W, Vaeteewoottacharn K, Sawanyawisuth K, Silsirivanit A, Kaewkong W, et al. Functional and genetic characterization of three cell lines derived from a single tumor of an *Opisthorchis viverrini*-associated cholangiocarcinoma patient. Hum Cell. 2020;33(3):695-708. doi: 10.1007/s13577-020-00334-w.
33. Jamnongsong S, Kueanjinda P, Buraphat P, Sakornsakolpat P, Vaeteewoottacharn K, Okada S, et al. Comprehensive drug response profiling and pan-omic analysis identified therapeutic candidates and prognostic biomarkers for Asian cholangiocarcinoma. iScience. 2022;25(10):105182. doi: 10.1016/j.isci.2022.105182.