Cytotoxic Effect of *Coscinium fenestratum* on Human Head and Neck Cancer Cell Line (HN31)

Saranyapin Potikanond, 1 Natthakarn Chiranthanut, 1 Parirat Khonsung, 1 and Supanimit Teekachunhatean 1,2

1 Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
2 Center of Thai Traditional and Complementary Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Correspondence should be addressed to Supanimit Teekachunhatean; supanimit.t@cmu.ac.th

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Introduction

Head and neck cancer is defined as cancer that arises in the head or neck regions including the nasal cavity, oral cavity, larynx, and pharynx. Most head and neck cancers are squamous cell carcinomas. Although various treatment options are available, including chemotherapeutic agents such as 5-fluorouracil (5-FU) and cisplatin, those agents have not dramatically improved overall five-year survival rates [1–3]. Two main reasons for that lack of improvement are resistance to classical cytotoxic agents [4] and rapid lymphatic dissemination and metastasis, both of which contribute to the poor prognosis [5]. Traditional anticancer agents, prepared mainly from natural herbs, represent a potentially effective alternative.

*Coscinium fenestratum*, or yellow vine, in the family Menispermaceae, a woody climber with yellow wood and sap, is widely used as a medicinal plant in many Southeast Asian countries [6–8] for fever, muscle pain, abdominal pain, inflammation [9], and malaria [10]. In addition, *C. fenestratum* has been reported to possess antioxidant [11], hypotensive [7, 12], antidiabetic [13], lipid lowering [14], antiplasmodal [10], and antibacterial [15] activities. Phytochemical studies have shown that the main alkaloidal constituent of *C. fenestratum* is berberine [16, 17] in addition to a smaller amount of protoberberine [6, 18]. The antineoplastic effects of berberine have been described in several studies [19, 20]. Berberine has been shown to inhibit the growth of tumor cell lines including cell lines of breast cancer [21], melanoma [22], liver cancer, and pancreatic cancer [23]. Moreover, berberine has been shown to have an antimutagenic effect on animal models [24] and to decrease the invasive properties of various tumors [25–35]. Other studies have demonstrated that berberine obtained from *C. fenestratum* has an antiproliferative effect on human nonsmall cell lung adenocarcinoma (NCI-H838 cell line) [36], colorectal cancer cells [37], and
acute myeloid leukemia (HL-60 cell line) [38]. Different cancer cells respond differently to *C. fenestratum* [39], but the effect of *C. fenestratum* on head and neck squamous cell carcinoma (HNSSC) has not yet been investigated. The objective of this study was to investigate the cytotoxic effect of a crude water extract of *C. fenestratum* compared to 5-FU on the human HNSSC cell line, HN31.

2. Materials and Methods

2.1. *C. fenestratum* Extract and 5-FU. The *C. fenestratum* used in this study was identified by the Faculty of Pharmacy and collected by the Faculty of Medicine, Chiang Mai University (voucher number PHCO-CM 028) [12]. The specimens were prepared from 100 g of dried *C. fenestratum* stems cut into thin pieces which were boiled in 500 mL of distilled water and then filtered. The procedure was repeated three times and the water extract was pooled and concentrated using a rotary evaporator at 70°C and then finally spray-dried yielding a total of 10 g of *C. fenestratum* extract (CF extract). The dried powder extract obtained was stored in a desiccator (25°C) until being used. The CF extract used in the experiment was freshly prepared by dissolving it in either distilled water or cell culture medium [12]. The 5-FU (or EFFCIL) used as a positive control for comparison of the cytotoxic effect with CF extract was obtained from Boryung Pharmaceutical Co. Ltd., Korea.

2.2. Cell Culture. The HNSSCC cell line HN31 was kindly provided by Associate Professor Dr. Prasit Pavasat (Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand) [40]. HN31 is a metastatic lymph node squamous cell carcinoma of the pharynx [41]. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine (Gibco, UK) supplemented with 10% fetal bovine serum (FBS), (Gibco, UK), 100 IU/mL penicillin, and 100 mg/mL streptomycin. The cells were cultured at 37°C in a humidified atmosphere with 5% CO₂. Cells were detached from the culture flask by treatment with 0.02% EDTA or 0.25% trypsin for 10 min. at 37°C and then subcultured when 70–80% confluence was reached, approximately every 2-3 days.

2.3. Cell Morphological Determination. Cells were incubated in either the absence or the presence of CF extract in serum-free medium for 48 h in 24-well plates. Morphology of cells was visualized using a light inverted microscope at 400x magnification and a digital camera.

2.4. Cell Viability Assay. Assay of the cytotoxic effect of the CF extract was performed using MTT dye (a yellow power of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) [40]. This colorimetric assay is a standard test for assessing cell viability. Living cells have NAD(P)H-dependent cellular oxidoreductase enzymes which reduce the MTT dye to insoluble formazan. Cells (1 × 10⁴ cells/well) were incubated on 96-well tissue culture plates in serum-free medium overnight. Different concentrations (0.01, 0.1, 2.5, 5, 10, and 25 mg/mL) of either CF extract or 5-FU were applied for 48 h. Following that, the medium was discarded and the cells were incubated with MTT solution for 4 h at 37°C and under 5% CO₂. The formazan crystals were solubilized by incubating the cells with 100% dimethyl sulfoxide (DMSO) and were immediately measured at a wavelength of 540 nm using a microplate reader. The concentration at which 50% cell growth inhibition occurred (IC₅₀) was calculated using a nonlinear curve fit tool (GraphPad Prism version 5). To determine whether a synergistic effect exists between 5-FU and CF extract, the cell lines were incubated with a mixture of CF extract at its IC₅₀ and various concentrations of 5-FU for 48 h under the same condition as aforementioned.

2.5. Determination of Apoptosis. The effect of CF extract on cell apoptosis was determined using an Annexin-V-FITC and propidium iodide kit (Invitrogen). Living cells which have intact plasma membranes are not stained by Annexin-V-FITC or propidium iodide fluorescent dye. However, when apoptosis occurs, phospholipid phosphatidylserine [42] is translocated from the inner to the outer leaflet and will be exposed and stained with Annexin-V-FITC. When cells are dead, DNA can be easily labeled with propidium iodide. Tests were accomplished according to the protocol provided by the manufacturer. 3 × 10⁵ cells in 24-well plates were starved in serum-free medium (SFM) overnight [43]. Then, either CF extract or 5-FU at different concentrations was added. After 18 h incubation, cells were trypsinized and washed two times in phosphate buffered saline (PBS). Binding buffer followed by Annexin-V-FITC and propidium iodide were applied in the dark for 10 minutes. The apoptotic and surviving fractions were determined by flow cytometry.

2.6. Gel Electrophoresis and Immunoblot Assays. Protein analysis by western blotting was used to explore various cellular mechanisms. Cells were incubated either with or without CF extract for 1 h. Proteins were extracted by lysis buffer (20 mM Tris-HCl, 1 mM Na3VO4, 5 mM NaF, and a cocktail of protease and phosphatase inhibitors) and extracted in 2x sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. The protein preparations were separated using 10% SDS-PAGE. The separated proteins were then transferred to PVDF membranes (Immobilon-P) for 1 h at 100 voltage [44]. After blocking with 5% nonfat dry milk/TBST, primary antibodies were incubated for either 2 h at room temperature or overnight at 4°C. Primary antibodies (anti-caspase-3, anti-Bax, anti-Bcl2, and anti-actin) were purchased from Santa Cruz Biotech, USA. Phosphospecific p38 MAPK, pAkt, and p53 were obtained from Cell Signaling, USA. All primary antibodies were used at concentrations of 1:1,000. The secondary antibody was horseradish peroxidase: conjugated goat anti-mouse or goat anti-rabbit antibody. Protein bands were visualized using the Amersham ECL detection reagents system (GE Healthcare, UK) and developed on Hyperfilm (GE Healthcare, UK) [45].

2.7. Statistical Analysis. All data was expressed as means ± SEM. Comparisons were made using one-way analysis of variance (ANOVA) with post hoc test and P values of <0.05 were regarded as statistically significant.
3. Results

3.1. Cytotoxic Effect of CF Extract on Cell Morphology. Normal HN31 is flat with a polygonal appearance (Figure 1(a)). After CF extract (at concentrations of 1 and 10 mg/mL) was applied for 48 h, the morphological changes observed included shrinkage and a shift to a rounded appearance (Figures 1(d)–1(f)).

3.2. Cytotoxic Effect of CF Extract on Cell Viability. MTT assay was used to evaluate the cytotoxic effect as nonviable cells cannot reduce or convert the MTT dye (tetrazolium dye) into insoluble formazan crystals. The intensity of the purple color was used to determine the ratio of viable cells. CF extract at a concentration of 1 mg/mL or higher significantly reduced the percentage of viable cells, while 5-FU showed significant reductions at all tested concentrations (Figure 2). Maximal effect with CF extract (approximately 80% reduction in dye intensity of control cultures) was achieved at 2.5 mg/mL, while increasing concentrations of 5-FU resulted in a more gradual decline, reaching maximal effect at 10 mg/mL. The IC$_{50}$ at 48-hour incubation of CF extract on HN31 was 0.12 mg/mL, while the IC$_{50}$ of 5-FU was 6.6 mg/mL, indicating that CF extract has a higher potency.

3.3. Combined Effect of CF Extract and 5-FU on Cell Viability. The dose of CF extract at IC$_{50}$ was applied to different concentrations of 5-FU, and then cell viability MTT assay was performed. The combination of CF extract and 5-FU in varying concentrations did not show significant reductions, indicating that CF extract and 5-FU do not have a synergistic effect (Figure 3).

3.4. The Effect of CF Extract on Cell Apoptosis. Apoptosis evaluation was performed using Annexin-V and propidium iodide assay followed by flow cytometry. Results of that assay,
shown in Figure 4, indicate the number of viable, apoptotic, and necrotic cells calculated based on the presence of phosphatidylserine (PS) molecules on the apoptotic cells. Viable cells with intact membranes and unexposed PS molecules remain unlabeled (cells in the 3rd quadrant or Q3). Propidium iodide enters the compromised membranes of necrotic cells and stains the DNA (cells in the 1st quadrant or Q1). Early apoptotic cells, with exposed PS molecules but without membrane abruption, are bound with Annexin-V (cells in the 4th quadrant or Q4). The cells in the 2nd quadrant (or Q2) are early necrosis, binding with both Annexin-V and propidium iodide. After 18 h of incubation, untreated cells (control) had low levels of apoptotic cell death, determined from the percentage of cells in Q4 (2.93 ± 0.64%). The CF extract at 1, 5, and 10 mg/mL significantly increased the percentage of apoptotic cell death in comparison to the control: 5.97 ± 0.61%, 9.1 ± 0.52, and 35.30 ± 0.35%, respectively. Similarly, the percentage of apoptotic cell death following application of 5-FU at 5 mg/mL was 18.03 ± 0.61% and was also significantly greater than the control (Figure 4(g)).

3.5. The Effect of CF Extract on Signaling Molecules. Growth inhibition of CF extract on the HN31 cell line was found to be associated with the activation of p38 mitogen-activated protein kinases (p38 MAPK) which regulates cell proliferation, differentiation, and apoptosis. The CF extract decreased the phosphorylation of p38 MAPK in a dose-dependent fashion but slightly increased the proapoptotic protein Bax (Figure 5). The tumor suppressor molecule, p53, was dose dependently increased by the CF extract, while the tumor survival molecule, pAkt, was decreased. These results indicate that a cytotoxic effect of CF extract is likely to be mediated at least through inhibiting survival signal molecules and increasing apoptotic proteins.

4. Discussion

Head and neck cancer cells are among the more aggressive tumors and are less sensitive to available anticancer agents. Drugs resistance [4] and metastasis [5] are major problems. This study choses to focus on HN31 because pharyngeal cancers are among the ten most common cancers in Thailand [46] and HN31 is a particularly aggressive lymphatic metastatic cell line [41], which often fails to respond adequately to conventional chemotherapy.

Several studies have shown that the stem of C. fenestratum has an antiproliferative effect against many cancers [37, 39]. Since the stems of C. fenestratum boiled in water have long been used in Thai traditional medicine, this research processed a water extract of C. fenestratum to investigate its cytotoxic effect on the HN31 cell line. This study found that CF extract resulted in a reduction in cell viability and increase in apoptosis in the HN31 cell line, but no synergistic effects of CF extract and 5-FU were observed. The cytotoxic effect was mediated via modulation of p38 MAPK, pAkt, and p53 proteins.

5-FU and crude CF extract investigated in this study had cytotoxic effect, and the dose at which 50% cell growth inhibition (IC50) occurs is a measure of the strength or potency of a given compound: the higher the IC50, the lower the potency. The IC50 of 5-FU for the HN31 cell line reported in the present study was 6.6 mg/mL, which is relatively high, presumably due to its mutation of the tumor suppressor gene p53 [41, 47, 48]. In contrast, our preliminary study found that the IC50 values of 5-FU for the less invasive HNSCC cell lines were substantially lower, for example, 10.26 μg/mL for HN30 and 24.98 μg/mL for HN22 (unpublished data), indicating the considerably decreased susceptibility of the HN31 cell line to 5-FU. This study also found that CF extract has a higher potency on HN31 (IC50 of 0.12 mg/mL) than 5-FU. Other studies, however, have reported that the IC50 of CF extract on lung-related tumor cells was about 0.001 mg/mL [39], that is, 100 times more potent. That difference in potency may be due to the HNSCC cell line being less susceptible to CF extract than lung cancer cells or to the fact that the lung cancer study used a methanol-water CF extract which may have had more of the active ingredient berberine [17]. The dry weight of berberine obtained from alcohol crude extracts in that study was 11.84–18.45% [17]. The yield of berberine from water CF extract used in this study as well as the cytotoxicity of berberine on HN31 should be investigated further.

The present study demonstrates that CF extract can decrease the survival and proliferation of the signal molecule pAkt which is key to 5-FU chemoresistance in squamous carcinoma cells [49]. In addition to the pAkt survival pathways, the mitogen-activated protein kinases (MAPKs) are also involved in cancer chemoresistance [50]. Most HNSCC exhibits activation of p38 MAPK [51]; therefore, the inhibitory effect of CF extract on pAkt and p38 MAPK activity possibly contributes to its efficacy in preventing or reducing cancer chemoresistance. Furthermore, it has been demonstrated that p53 is another crucial protein that plays an important role in tumor suppression and tumorigenesis [48]. Therefore, if CF extract can increase the expression of
p53, it could be expected that its mechanism of action is also mediated via increased tumor suppression.

Combination in cancer therapy has been standard care aiming at maximizing efficacy while minimizing systemic toxicity [52]. Each of the drugs carries its own risks and benefits. For example, 5-FU has many side effects such as myelosuppression and cardiotoxicity [53–55]. Previous research by the authors found that high doses of CF water extract did not have a toxic effect or significantly change any other parameters in rats [12]. Another study demonstrated
that CF alcoholic extract does have a neurotoxic effect on rats [56]. This discrepancy might be due to the difference in the extraction solutions used. The maximal anticancer efficacy of CF extract and 5-FU used individually was comparable; however, using crude CF extract and 5-FU in combination did not have a synergistic effect. In general, the principles of combination therapy for cancer are primarily based on using drug with nonoverlapping toxicities and combining agents with different mechanism of action [52]. The reason for the lack of synergism was not immediately evident; it is postulated that the two substances might possess the similar mechanism of action or exert the similar modulation on signaling molecules of HN31. This experimental result argues against the use of a combination of crude CF extract with 5-FU in a clinical setting.

Some limitations regarding this study should be addressed. First, the active ingredient(s) of CF extract (e.g., berberine and others) have not been quantified. Second, the cytotoxic effect of CF extract on other HNSCC cell lines has not been studied. Third, the effect of CF extract on other signaling molecules (e.g., NF-kappaB signaling, PI3K/mTOR, and NOTCH, intercellular adhesion molecule-1 (ICAM-1)) involved in tumorigenesis and metastasis [57–59] has not been fully understood and warrants further investigation.

5. Conclusions

CF extract has potential as chemotherapeutic agent. Its cytotoxicity was associated with the modulation of p38 MAPK, pAkt, and p53 signal molecules, leading to inhibiting cell survival and increasing apoptosis. No synergistic effects of CF extract and 5-FU were observed.

Conflict of Interests

There is no conflict of interests. None of the authors has a direct financial relation with any of the commercial identities mentioned in the paper.

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References

[1] E. E. Vokes, “Induction chemotherapy for head and neck cancer: recent data,” The Oncologist, vol. 15, supplement 3, pp. 3–7, 2010.
[2] D. Mukherjea and L. P. Rybak, “Pharmacogenomics of cisplatin-induced ototoxicity,” Pharmacogenomics, vol. 12, no. 7, pp. 1039–1050, 2011.
[3] T. Yu, R. E. Wood, and H. C. Tenenbaum, “Delays in diagnosis of head and neck cancers,” Journal of the Canadian Dental Association, vol. 74, no. 1, article 61, 2008.
[4] C. Holohan, S. van Schaeybroeck, D. B. Longley, and P. G. Johnston, “Cancer drug resistance: an evolving paradigm,” Nature Reviews Cancer, vol. 13, no. 10, pp. 714–726, 2013.
[5] J. Massano, F. S. Regateiro, G. Januário, and A. Ferreira, “Oral squamous cell carcinoma: review of prognostic and predictive factors,” Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology, vol. 102, no. 1, pp. 67–76, 2006.
[6] P. M. M. Pinho, M. M. M. Pinto, A. Kijjoa, K. Pharadai, J. G. Díaz, and W. Herz, “Protoberberine alkaloids from Coscinium fenestratum,” Phytochemistry, vol. 31, no. 4, pp. 1403–1407, 1992.
[7] G. B. Singh, S. Singh, S. Bani, and S. Malhotra, “Hypotensive action of a Coscinium fenestratum stem extract,” Journal of Ethnopharmacology, vol. 30, no. 2, pp. 151–155, 1990.
[8] J. Siwon, R. Verpoorte, G. F. A. van Essen, and A. Baerheim Svendsen, “Studies on Indonesian medicinal plants. III: the alkaloids of Coscinium fenestratum,” Planta Medica, vol. 38, no. 1, pp. 24–32, 1980.
[9] S. Sudharshan, T. Prasith Kekuda, and M. Sujatha, “Antinflammatory activity of Curcuma aromatica Salis and Coscinium fenestratum Colebr: a comparative study,” Journal of Pharmacy Research, vol. 3, no. 10, pp. 24–25, 2010.
[10] Q. L. Tran, Y. Tezuka, J. Y. Ueda et al., “In vitro antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine,” Journal of Ethnopharmacology, vol. 86, no. 2-3, pp. 249–252, 2003.
[11] M. R. Venukumar and M. S. Latha, “Antioxidant effect of Coscinium fenestratum in carbon tetrachloride treated rats,” Indian Journal of Physiology and Pharmacology, vol. 46, no. 2, pp. 223–228, 2002.
[12] T. Wongcome, A. Panthong, S. Jasdanont, D. Kanjanapothi, T. Taesotikul, and N. Lertprasatsin, “Hypotensive effect and toxicology of the extract from Coscinium fenestratum (Gaertn.) Colebr.”, Journal of Ethnopharmacology, vol. 111, no. 3, pp. 468–475, 2007.

[13] A. Shirwaikar, K. Rajendran, and I. S. R. Punitha, “Antidiabetic activity of alcoholic stem extract of Coscinium fenestratum in streptozotocin-nicotinamide induced type 2 diabetic rats,” Journal of Ethnopharmacology, vol. 97, no. 2, pp. 369–374, 2005.

[14] W. Jittaprasatsin, V. Banlunara, D. Sommitr, and J. Tang, “Berberine inhibits the growth of Anoikis-resistant MCF-7 and MDA-MB-231 human breast cancer cells,” Thai Journal of Pharmacology, vol. 27, pp. 109–120, 2005.

[15] G. M. Nair, S. Narasimhan, S. Shiburaj, and T. K. Abraham, “Antibacterial effects of Coscinium fenestratum,” Fitoterapia, vol. 76, no. 6, pp. 585–587, 2005.

[16] P. Rojsanga, W. Gritsanapan, and L. Suntronsuk, “Determination of berberine content in the stem extracts of Coscinium fenestratum by TLC densitometry,” Medical Principles and Practice, vol. 15, no. 5, pp. 373–378, 2006.

[17] P. Rojsanga and W. Gritsanapan, “Variation of berberine content in coscinium fenestratum stem in Thailand Market,” The Mahidol University Journal of Pharmaceutical Sciences, vol. 32, no. 3–4, pp. 66–70, 2005.

[18] S. Malhotra, S. C. Taneya, and K. L. Dhar, “Minor alkaloid from Coscinium fenestratum,” Phytochemistry, vol. 28, no. 7, pp. 1998–1999, 1989.

[19] J. Tang, Y. Feng, S. Tsao, N. Wang, R. Curtain, and Y. Wang, “Berberine and coptidis rhizoma as novelantineoplastic agents: a review of traditional use and biomedical investigations,” Journal of Ethnopharmacology, vol. 126, no. 1, pp. 5–17, 2009.

[20] Y. Sun, K. Xun, Y. Wang, and X. Chen, “A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs,” Anti-Cancer Drugs, vol. 20, no. 9, pp. 757–769, 2009.

[21] J. B. Kim, J. H. Yu, E. Ko et al., “The alkaloid Berberine inhibits the growth of Anoikis-resistant MCF-7 and MDA-MB-231 breast cancer cell lines by inducing cell cycle arrest,” Phytomedicine, vol. 17, no. 6, pp. 436–440, 2010.

[22] T. L. Serafin, P. J. Oliveira, V. A. Sardao, E. Perkins, D. Parke, and J. Holy, “Different concentrations of berberine result in distinctcellular localization patterns and cell cycle effects in a melanoma cell line,” Cancer Chemotherapy and Pharmacology, vol. 61, no. 6, pp. 1007–1018, 2008.

[23] L. Pinto-Garcia, T. Effert, A. Torres, J. D. Hoheisel, and M. Youns, “Berberine inhibits cell growth and mediates caspase-dependent cell death in human pancreatic cancer cells,” Planta Medica, vol. 76, no. 11, pp. 1155–1161, 2010.

[24] G. Sindhu and S. Manoharan, “Anti-clastogenic effect of berberine against DMBA-induced clastogenesis,” Basic and Clinical Pharmacology and Toxicology, vol. 107, no. 4, pp. 818–824, 2010.

[25] M. K. Pandey, B. Sung, A. B. Kunnumakkara, G. Sethi, M. M. Chaturvedi, and B. B. Aggarwal, “Berberine modifies cytokine 179 of IL8 kinase, suppresses nuclear factor-kB-regulated antiapoptotic gene products, and potentiates apoptosis,” Cancer Research, vol. 68, no. 13, pp. 5370–5379, 2008.

[26] J. B. Kim, E. Ko, W. Han, I. Shin, S. Y. Park, and D.-Y. Non, “Berberine diminishes the side population and ABCG2 transporter expression in MCF-7 breast cancer cells,” Planta Medica, vol. 74, no. 14, pp. 1693–1700, 2008.

[27] S. Kim, J. H. Choi, J. B. Kim et al., “Berberine suppresses TNF-alpha-induced MMP-9 and cell invasion through inhibition of AP-1 activity in MDA-MB-231 human breast cancer cells,” Molecules, vol. 13, no. 12, pp. 2975–2985, 2008.

[28] C. J. Thirupurasundari, R. Padmini, and S. N. Devaraj, “Effect of berberine on the antioxidant status, ultrastructural modifications and protein bound carbohydrates in azoxymethane-induced colon cancer in rats,” Chemico-Biological Interactions, vol. 177, no. 3, pp. 190–195, 2009.

[29] S. K. Mantena, S. D. Sharma, and S. K. Katiyar, “Berberine, a natural product, induces G2-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells,” Molecular Cancer Therapeutics, vol. 5, no. 2, pp. 296–308, 2006.

[30] S. B. Muralimanoharan, A. B. Kunnumakkara, B. Shylesh et al., “Butanol fraction containing berberine or related compound from Nuxretine inhibits NFkB signaling and induces apoptosis in prostate cancer cells,” The Prostate, vol. 69, no. 5, pp. 494–504, 2009.

[31] M. S. Choi, J. H. Oh, S. M. Kim et al., “Berberine inhibits p53-dependent cell growth through induction of apoptosis of prostate cancer cells,” International Journal of Oncology, vol. 34, no. 5, pp. 1221–1230, 2009.

[32] G.-Y. Wang, Q.-H. Lv, Q. Dong, R.-Z. Xu, and Q.-H. Dong, “Berbamine induces fas-mediated apoptosis in human hepatocellular carcinoma HepG2 cells and inhibits its tumor growth in nude mice,” Journal of Asian Natural Products Research, vol. 11, no. 3, pp. 219–228, 2009.

[33] C.-C. Lin, L. T. Ng, F.-F. Hsu, D.-E. Shieh, and L.-C. Chiang, “Cytotoxic effects of Coptis chinensis and Epimedium sagittatum extracts and their major constituents (berberine, coptisine and icariin) on hepatoma and leukaemia cell growth,” Clinical and Experimental Pharmacology & Physiology, vol. 31, no. 1-2, pp. 65–69, 2004.

[34] S. C. Myoung, Y. Y. Dong, H. O. Ju et al., “Berberine inhibits human neuroblastoma cell growth through induction of p53-dependent apoptosis,” Anticancer Research, vol. 28, no. 6, pp. 3777–3784, 2008.

[35] C.-C. Lin, S.-Y. Lin, J.-G. Chung, J.-P. Lin, G.-W. Chen, and S.-T. Kao, “Down-regulation of cyclin Bi and Up-regulation of Weel by berberine promotes entry of leukemia cells into the G2/M-phase of the cell cycle,” Anticancer Research, vol. 26, no. 2A, pp. 1097–1104, 2006.

[36] R. Tungradit, S. Sinchaikul, S. Phutrakul, W. Wongkham, and S.-T. Chen, “Antiproliferative activity of berberine from Coscinium fenestratum on NCI-H1838 cell line,” Chiang Mai Journal of Science, vol. 38, no. 1, pp. 85–94, 2011.

[37] P. Rojsanga, M. Sukthhankar, C. Krisanapun, W. Gritsanapan, D. B. Lawson, and J. S. Back, “In vitro anti-proliferative activity of alcoholic stem extract of coscinium fenestratum in human colorectal cancer cells,” Experimental and Therapeutic Medicine, vol. 1, no. 1, pp. 181–186, 2010.

[38] R. Tungradit, S. Supachok, S. Phutrakul, W. Wongkham, and S.-T. Chen, “Anti-cancer compound screening and isolation: Coscinium fenestratum, Tinospora crispa and Tinospora cordifolia,” Chiang Mai Journal of Science, vol. 37, no. 3, pp. 476–488, 2010.

[39] J.-Y. Ueda, Y. Tezuka, A. H. Banskota et al., “Antiproliferative activity of Vietnamese medicinal plants,” Biological & Pharmaceutical Bulletin, vol. 25, no. 6, pp. 753–760, 2002.

[40] R. Kaomongkolgit, N. Chaisomboon, and P. Pavasant, “Apoptotic effect of alpha-mangostin on head and neck squamous
carcinoma cells," Archives of Oral Biology, vol. 56, no. 5, pp. 483–490, 2011.

[41] D. Sano, T.-X. Xie, T. J. Ow et al., "Disruptive TP53 mutation is associated with aggressive disease characteristics in an orthotopic murine model of oral tongue cancer," Clinical Cancer Research, vol. 17, no. 21, pp. 6658–6670, 2011.

[42] S. J. Martin, C. P. M. Reutelingsperger, A. J. McGathon et al., "Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: Inhibition by overexpression of Bcl-2 and Abl," The Journal of Experimental Medicine, vol. 182, no. 5, pp. 1545–1556, 1995.

[43] W. M. Abuzeid, S. Davis, A. L. Tang et al., "Sensitization of head and neck cancer to cisplatin through the use of a novel curcumin analog," Archives of Otolaryngology—Head & Neck Surgery, vol. 137, no. 5, pp. 499–507, 2011.

[44] H. Towbin, T. Staehelin, and J. Gordon, "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications," Proceedings of the National Academy of Sciences of the United States of America, vol. 76, no. 9, pp. 4350–4354, 1979.

[45] W. Pratchayasakul, S. Kerdphoo, P. Petsophonsakul, A. Pongchaidecha, N. Chattipakorn, and S. C. Chattipakorn, "Effects of high-fat diet on insulin receptor function in rat hippocampus and the level of neuronal corticosterone," Life Sciences, vol. 88, no. 13-14, pp. 619–627, 2011.

[46] H. Sriplung, S. Sontipong, N. Martin et al., "Cancer incidence in Thailand, 1995–1997," Asian Pacific Journal of Cancer Prevention, vol. 6, no. 3, pp. 276–281, 2005.

[47] W. A. Yeudall, J. Jakus, J. F. Ensley, and K. C. Robbins, "Functional characterization of p53 molecules expressed in human squamous cell carcinomas of the head and neck," Molecular Carcinogenesis, vol. 18, no. 2, pp. 89–96, 1997.

[48] Z. P. Pavelic and J. L. Gluckman, "The role of p53 tumor suppressor gene in human head and neck tumorigenesis," Acta Oto-Laryngologica, Supplement, vol. 527, pp. 21–24, 1997.

[49] F. You, K. Aoki, Y. Ito, and S. Nakashima, "AKT plays a pivotal role in the acquisition of resistance to 5-fluorouracil in human squamous carcinoma cells," Molecular Medicine Reports, vol. 2, no. 4, pp. 609–613, 2009.

[50] W. Jin, L. Wu, K. Liang, B. Liu, Y. Lu, and Z. Fan, "Roles of the PI-3K and MEK pathways in Ras-mediated chemoresistance in breast cancer cells," British Journal of Cancer, vol. 89, no. 1, pp. 185–191, 2003.

[51] K. Leelahavanichkul, P. Amornphimoltham, A. A. Molinolo, J. R. Basile, S. Koontongkaew, and J. S. Gutkind, "A role for p38 MAPK in head and neck cancer cell growth and tumor-induced angiogenesis and lymphangiogenesis," Molecular Oncology, vol. 8, no. 1, pp. 105–118, 2014.

[52] A. C. Pinto, J. N. Moreira, and S. Simões, Current Cancer Treatment—Novel Beyond Conventional Approaches, InTech, 2011.

[53] D. Vyas, G. Laput, and A. Vyas, "Chemotherapy-enhanced inflammation may lead to the failure of therapy and metastasis," Oncotargets and Therapy, vol. 7, pp. 1015–1023, 2014.

[54] H. Broder, R. A. Gottlieb, and N. E. Lepor, "Chemotherapy and cardiotoxicity," Reviews in Cardiovascular Medicine, vol. 9, no. 2, pp. 75–83, 2008.

[55] S.-E. Al-Batran and J. A. Ajani, "Impact of chemotherapy on quality of life in patients with metastatic esophagogastric cancer," Cancer, vol. 116, no. 11, pp. 2511–2518, 2010.