

CULTIVATING PROMISING ANTICANCER TREATMENT FOR COLORECTAL CANCER WITH ESCO CELCULTURE® CO₂ INCUBATOR

Abstract

Despite the various available chemotherapy treatment, colorectal cancer remains as one of the common causes of cancer-related death around the world, affecting both men and women of all racial and ethnic groups. One novel approach to curing colorectal cancer was reported by a recent study which tapped the potential of *Curcuma mangga* rhizome extracts as anticancer agents against human colorectal cancer cells. Human colorectal adenocarcinoma (HT29) and normal colon (CCD-18Co) cell lines were treated with increasing dose of hexane (CMH) and ethyl acetate (CME) extracts and were then cultured in an Esco CelCulture® CO₂ incubator with increasing incubation period. Both CMH and CME extracts showed increasing cytotoxic effects against HT29 cell lines in a dose- and time-dependent manner. Given the favorable outcome of the study, CelCulture® persists as a reliable tool in the pursuit of a promising cure against colorectal cancer.

Introduction

Cancer beginning in the epithelial lining of the colon or rectum is known as colorectal cancer.¹ It is formed when the normal cell of the colon or rectum undergoes a series of mutations in its specific DNA sequences, thereby affecting its normal proliferation and self-renewal mechanism.² It accounts for ten percent of cancer worldwide in 2008 and 2012 and is regarded as the second leading cause of cancer-related deaths in the United States, affecting both men and women of all racial and ethnic groups, often at the age of 50 years or older.³ The risk of colorectal cancer is associated with alcohol intake, obesity, smoking, consumption of processed and red meat, inflammatory bowel disease, family history of colorectal cancer, and age.⁴ Currently, the treatment for colorectal cancer typically involves surgery, chemotherapy, radiation therapy, and combined treatment.⁵

Chemoprevention

One novel approach in controlling cancer is called chemoprevention which involves utilizing specific natural or synthetic products to reverse or suppress the development of cancer.⁶ Since most colorectal cancers are adenocarcinomas, or cancers from cells that generate mucus and other fluids¹, they are one of the targets in identifying the efficacy of promising chemopreventive agents.⁷ A potential natural approach to chemoprevention is using extracts from a plant called *Curcuma mangga* (*C. mangga*). *C. mangga*, also known as “mango-like turmeric”, is a rhizomatous herb from the family of Zingiberaceae and is being used as a medicine and spice in Indonesia and Malaysia.⁸ Numerous previous research have reported the cytotoxic and antiproliferation effects of *C. mangga* on colorectal carcinoma and many other types of carcinoma. An investigation on its mechanism of inducing apoptosis in cancer cell lines was pioneered by Hong et al. (2016) in their study entitled, “*Non-aqueous extracts of Curcuma mangga rhizomes induced cell death in human colorectal adenocarcinoma cell line (HT29) via induction of apoptosis and cell cycle arrest at G₀/G₁ phase.*”⁹ In the study, the cytotoxic activity of hexane (CMH) and ethyl acetate (CME) extracts of *C. mangga* rhizomes on human colorectal adenocarcinoma cell lines (HT29) and human normal colon cell lines (CCD-18Co) was determined through SRB assay and morphological assessment. Throughout the research, the cell lines tested were incubated and

¹ National Cancer Institute

² Fanali et al., 2014

³ Centers for Disease Control and Prevention, 2017

⁴ Johnson et al., 2014

⁵ DeSantis et al., 2014

⁶ Rajamanickam and Agarwal, 2008

⁷ Hawk and Levin, 2005

⁸ Malek et al., 2011

⁹ Hong et al., 2016

maintained under a constant humidified condition with 5.0% CO₂ at 37°C in Esco CelCulture® CO₂ Incubator (Model: CCL-170B-8) with varying duration of incubation.

SRB Cytotoxicity Assay

HT29 and CCD-18Co were tested with different dose (20, 30, and 40 µg/mL) of CMH and CME extracts and incubated for different periods (24, 48, and 72 hours) in Esco CelCulture® CO₂ incubator. Both extracts showed increasing cytotoxic effect against HT29 cells in a dose- and time-dependent manner. On the other hand, results show that both extracts had a relatively mild toxic effect on CCD-18Co.⁹

Morphological Assessment

Signs of morphological changes associated with apoptosis such as cell shrinking, membrane blebbing, and formation of apoptotic bodies were evident in treated HT29 cells. Cells exposed to both extracts and incubated for 24, 48, and 72 hours have shown cell detachment from the bottom surface of the culture dish and further had DNA condensation and fragmentation. Early and late apoptosis in treated HT29 cells were detected by the positive result in Annexin-V staining in a dose- and time-dependent manner. Analysis on cell cycle distribution of treated HT29 cells further revealed that the cells were arrested in the G₀/G₁ phase in which cells may either enter repair phase or undergo apoptosis.⁹

CelCulture® CO₂ Incubator

The favorable outcome of the tests to determine the cytotoxic effects of CMH and CME on HT29 and CCD-18Co cells was made possible through the consistently stable environment provided by Esco CelCulture® CO₂ Incubator. At a growth condition of humidified 5.0% CO₂ atmosphere at 37°C, the CelCulture® proved to be a dependable tool for achieving scientific goals, be it propagating cell lines prior to testing, incubating cells for cytotoxic assays, and preparing treated cells for morphological analysis.⁹

The precise parameter control with rapid recovery after door opening in CelCulture® is achieved by its direct heat and air jacketing design, combined with the advanced technology of its CARBOCAP® infrared CO₂ sensor and NTC temperature sensor, all controlled with high accuracy by the microcontroller PI system. Its VentiFlow™ forced convection feature allows for uniform distribution of parameters at all points inside the chamber while accelerating the humidification process to prevent the dehydration of cells. In addition, CelCulture® is equipped with redundant contamination control methods for maximum protection of the incubated cells. The air inside is continuously purified by the ULPA filtration system which efficiently filters 0.1 to 0.2 µm particulates at 99.999% efficiency, keeping the chamber in an ISO Class 4 clean atmosphere during normal operations. Its gas injection lines are also equipped with 0.2 µm in-line filters to remove impurities from gas tanks when CO₂ gas is injected into the chamber. Moreover, its external electro-galvanized steel surface is powder-coated with ISOCIDE™ silver ion-impregnated antimicrobial coating to eliminate 99.9% of surface contaminants within 24 hours of exposure. Furthermore, CelCulture® comes with an automated 90°C moist heat decontamination cycle which is proven to be effective against common laboratory contaminants with a 6 log reduction of bacterial spores and vegetative cells, and a 4 log reduction for fungal spore. Chamber is clean and dry after the decontamination cycle, eliminating the need for a further wipe down which may reintroduce room air contaminants into the chamber.



Fig. 1. The CelCulture® CO₂ Incubator used to determine the cytotoxic effects of hexane and ethyl acetate extracts on human colorectal adenocarcinoma cell lines and human normal colon cell lines. Model: CCL-170B-8

Conclusion

Increasing deaths due to colorectal cancer is still evident across the globe despite the various existing therapeutic methods currently available. *Curcuma mangga* extracts, hexane and ethyl acetate, proved to be a promising treatment for colorectal cancer with their ability to induce cell death in human colorectal adenocarcinoma cell lines and arrest them in the G₀/G₁ phase of cell cycle. Any further investigations on its anticancer mechanism against HT29 cell lines could be done, utilizing Esco's CelCulture® CO₂ incubator with its stable environmental conditions and complete contamination control methods to ensure efficient outcomes in finding novel approaches to treat colorectal cancer.

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