Research Article

Expression of Apoptosis Marker Genes bax and bcl-2 in WiDr Colon Cancer Cells Treated with Red Eye Sea Snail (*Cerithidea obtusa*) Extract

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Abstract | One of the characteristics of cancer is failure to regulate apoptosis process. Apoptosis is a programmed cell death that occur in every multicelluler organism. Bax and Bcl-2 are two proteins that are involved in controlling the apoptosis process. This research aimed to quantify the expression of bax and bcl-2 gene in colon cancer cell WiDr treated with red snail ethanol extract 125, 62.5 and 31.25 ppm concentration. mesenggerRNA (mRNA) was isolated from WiDr cells using a commercial kit. The mRNA concentration was then measured with a UV-Vis microvolume spectrophotometer at 260/28 nm. Relative expression of bax and bcl-2 to house keeping gene GAPDH was measured on a real time PCR machine. Relative gene expression was calculated using the ΔΔCt method. The result showed that bax gene expression increases 4.36, 4.77 and 4.63 times in 125, 62.5 and 31.25 ppm concentration, respectively. The expression of bcl-2 gene was decreased after treatment of red eye snail ethanol extract by 0.17, 0.16 and 0.14 in 125, 62.5 and 31.25 ppm extract concentration, respectively. Relatively, the bax:bcl-2 ratio was also increase. Red eye sea snail extract has a potential as alternative therapy on cancer by inducing apoptosis in WiDr colon cancer cells by controlling the expression of the genes bax and bcl-2. However, more research is needed to determine the processes governing bax and bcl-2 gene expression, and the safety of using red eye sea snail extract as an anti-cancer.

Keywords | Red eye snail, Apoptosis, Bax, Bcl-2, WiDr cells

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INTRODUCTION

Apoptosis is the term used to describe a natural process of cell death that takes place within the body. The capacity of cancer cells to evade or slow the process of apoptosis allows them to continue to divide and spread throughout the body. Tumorigenesis is a slow-moving process in which altered cells survive apoptosis as well as proliferate abnormally. Since the main cytotoxic mechanism of anticancer therapy is the stimulation of apoptosis, apoptosis inhibition is a critical stage in tumor growth but can also lead to therapeutic resistance (Lomperta et al., 2020). Apoptosis was also enhanced, primarily by impacting bax and bcl-xl, but also bcl-2 and p53 to a smaller extent. Furthermore, the combination of 5-Fluorouacil (5-FU) and Verbascoside (VER) significantly reduced PI3K and p-AKT/total AKT ratios (Attia et al., 2018).

Apoptosis marker genes are those that regulate the apoptotic process. Caspase, bcl-2, and bax are examples of apop-

tosis marker genes. Cancer cells frequently have mutations in apoptosis marker genes, making them resistant to the apoptotic process. N-acetylcysteine (NAC) also inhibits apoptosis by decreasing mRNA expression of bax-1, bax, and caspase-3 and increasing mRNA expression of bcl-2 (Zhuang et al., 2019). Quercetin inhibits colorectal cancer cell growth by increasing tumor supressor genes and modulating the cell growth and apoptosis-related genes (Shang et al., 2018).

Bcl-2 is a member of the bcl protein complex that is important for controlling the apoptosis process. Bcl-2 can block or inhibit pro-apoptosis proteins like bax, thereby halting the apoptosis process. Prior research suggests that GEN can substantially increase chemosensitivity by inhibiting cytosolic bcl-2 allocation and the relevance of bcl-2 and Beclin-1 in NSCLC cells (Zhang et al., 2018). The authors Marquez-Jurado et al. (2018) showed that mitochondrial mass serves as a proxy for apoptosis commitment. Interestingly, none of the medicines show any appreciable efficacy when used alone, combinations that targeted MCL-1 with bcl-2 and, to a smaller amount, bcl-2 demonstrated strong synergistic killing action induced by bak and bax. BFL-1 is not a crucial pro-survival protein in melanoma, that genetically induced deletion of it had little effect on the sensitivity to the BH3 mimic medication in cell lines expressing it at reasonably high levels. While the combination with the proteasome inhibitor bortezomib was more successful in several cell lines, the MCL-1 inhibitors and BRAF inhibitors only modestly increased the amount of melanoma cells killed on each treatment alone. According to the research, treatments that specifically target certain combinations of the pro-survival protein bcl-2, such as mcl-1 plus bcl-2 and mcl-1 plus bcl-2, may be highly effective treatment against melanoma (Lee et al., 2019).

Antioxidants and anti-inflammatory components found in red eye sea snail (*Cerithidea obtusa*) extract, as well as other bioactive substances, may have an impact on cancer cells. The study of red eye snail extract as anticancer has previously done before. Red eye snail extract has anti-proliferation activity against Hela (90,62%), A549 (79,84%) and K562 (76,71%) cells (Purwaningsih et al., 2008). Research by Purwaningsih (2012) shows that red eye snail extract has strong antioxidant activity with Inhibition Concentration (IC_{50}) 58,19 ppm. Anticancer activity, especially study on apoptosis gene expression of red eye snail extract on colon cancer cell WiDr have not been studied previously. The research aimed to quantify the expression of bak and bcl-2 gene in colon cancel cell WiDr treated with red eye snail extract. The findings of this study may offer fresh perspectives on the creation of natural cancer treatments. The findings also could provide significant additional data to the development of matah merah snail extract as anticancer.

MATERIALS AND METHODS

The research was carried out at the Microbiology and Immunology Laboratory, Centre for Primate Animal Research, IPB University Bogor, from October through December 2022.

Cell preparation

The WiDr colon cancer cell (ATCC CCL-218) used in this study is a collection of the Microbiology and Immunology Laboratory of the Center for Primate Animal Research, IPB University. WiDr cell cultures were grown in RPMI 1640 (Gibco) growth medium containing 10% v/v Fetal Bovine Serum (FBS) (Gibco) and 1% v/v penicillin-streptomycin (Gibco). WiDr cancer cells ($1x10^6$) were cultured in a 75 ml tube. After the cell density reached 50% confluency, the culture was further incubated for 24 hours with or without red eye extract treatment. At the end of the incubation period, cells were harvested using trypsin.

mRNA extraction and qPCR analysis

Total mRNA extraction. mRNA was isolated from WiDr cells using a commercial kit (Rneasy Mini Kit – QIAGEN) following company procedures. The mRNA concentration was then measured with a UV-Vis microvolume spectrophotometer (NanoDrop One 3300 –Thermo Scientific) at 260/28 nm.

Relative expression of bax and Bcl2 to house keeping gene GAPDH was measured on a real time PCR machine (iCycler w/ MyiQ_BioRad). The primers used for gene amplification are bak R 5'-CCTGCTAACCCTGAGATG-3', bak F 5'-TGGGGTCTCTACGCAAAC-3', bcl-2 R 5'-ATGGCAGCAGTAAAGCAAG-3', bcl-2 F 5'-GCTGCATTGTTCCCATAGA-3', and the gateway keeper gene GAPDH R 5'-TACGGCCAGAGGCGTACA-3', F 5'-TGACCCAGATCATGTTTG-3'. The reaction took place under conditions: 50 °C, 10 minutes for reverse transcriptase activation, 95 °C, 5 minutes for reverse transcriptase inactivation, then the reaction was repeated 40 cycles at 95 °C, 10 seconds for denaturation, 52 °C, 10 seconds for annealing, and 72 °C, 10 seconds for elongation. Baseline and threshold are set automatically by the software included in the machine. The intersection of the amplification curve with the value is the Cycle threshold (Ct). All samples were normalized to GAPDH gene expression and expressed as fold change. Relative gene expression was calculated using the ΔΔCt method (Pfaffl et al., 2002).

RESULTS AND DISCUSSION

Result

Bax and bcl-2 gene expression using real-time PCR method on WiDr cell line treated and untreated red eye snail extract presented in Figure 1 and Figure 2. mRNA used as a template for cDNA synthesis and amplification, later the result was monitored real time using real time PCR.

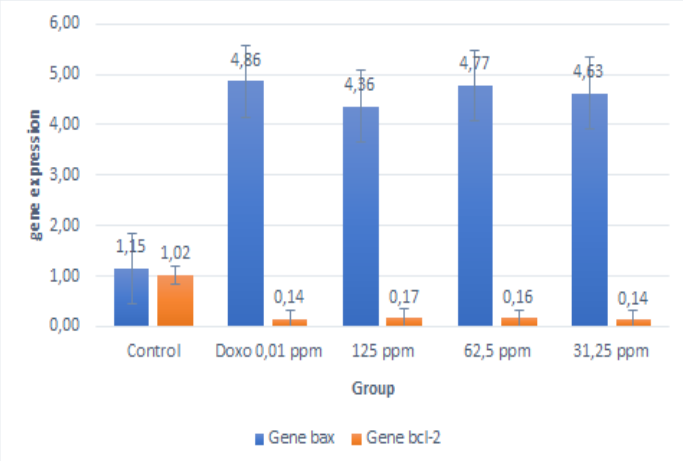

Figure 1: Apoptotic gene expression in WiDr cells treated with red eye snail extract at 125 ppm, 62.5 ppm, and 31.25 ppm. Positive control using doxorubicin 0.01 ppm. Blue color: bax gene, red color: bcl-2 gene.

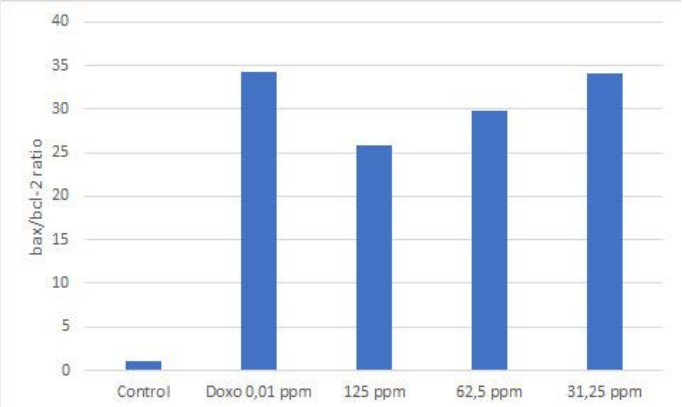

Figure 2: Ratio between pro (bax) and anti (bcl-2) apoptotic genes of WiDr cell treated with red eye snail extract.

The result in Figure 1 showed that the treatment group has higher bax gene exspression. The expression of bax gene increases 4.36 times in 125 ppm concentration, 4.77 times in 62.5 ppm concentration and 4.63 ppm concentration. Figure 1 also showed that the expression of bcl-2 gene was decreased after treatment of red eye snail extract by 0.17, 0.16 and 0.14 in 125, 62.5 and 31.25 ppm extract concentration, respectively. Relatively, tha bax : bcl-2 ratio was also increased. Figure 2 showed the ratio between pro and anti apoptosis gene. The real-time PCR method offers advantages over other methods like Northern blot or microarray because it requires fewer samples and is more sensitive and specific at detecting changes in gene expression. Real-time PCR can also be used to detect genetic polymorphisms or gene mutations.

DISCUSSION

Cell death regulation is one of the key mechanisms in cell growth control and cancer prevention, is regulated by genes known as apoptosis markers. Cancer cells frequently have altered or dysfunctional genes related with apoptosis, which permits cancer cells to continue to grow and proliferate. The bax and bcl-2 genes are two significant apoptotic indicators. The bcl-2 gene is anti-apoptotic, whereas the bax gene is pro-apoptotic, which promotes cell death. Changes in gene expression that are heritable, adaptive, and reversible are known as epigenetic events (Rajan et al., 2020). Moreover, they play an important role in gene function and expressed in damaged sensory neurons (Martin et al., 2019).

In this study, bax and bcl-2 gene expression analysis conducted using real time PCR in WiDr cell treated with red eye snail extract at concentrations of 125, 62.55, and 31.25 ppm to see the effect of the treatment. Our result showed that the extract treatment group of WiDr cells has significantly increased bak gene expression and decreased bcl-2 expression. The ratio of pro and anti apoptotic gene expression was also significantly increased in this study. This result showed that the proapoptotic gene was higher than antiapoptotic gene. Real-time PCR methods for gene expression analysis entail amplifying specific genes or RNA targets using fluorescent probes that emit signals that can be monitored in real-time throughout the amplification cycle. A relative change in gene expression is calculated by comparing the amount of the target gene or RNA to an internal control. The increase in bax:bcl-2 ratio means there are more bax gene expression than bcl-2 so the apoptotic process became higher in cell. Bax and bcl-2 ratio has a negative correlation against output chemotherapy of patient with chronic leucoblastic and it can be used as therapy prognosis marker (Supraptiningsih et al., 2019).

Although the mechanism needs to be studied more, but we presumed that active compound in red eye snail extract has the ability to induce apoptosis in cancer cells by controlling the ratio of pro and anti apoptotic gene. Antiproliferation activity of certain active compound in natural resources has been studied before. Previous research indicates that targeting dual binders individually or as part of modular proteins to cancer in the type of protein, mRNA, or DNA cancer cell apoptosis (Kim et al., 2022). MOMP causes cytoplasmic cytochrome c depletion, which starts the apoptotic chain reactions. When bak and bax are ab-

sent, intrinsic pathways initiate apoptosis (van Delft et al., 2019). White tea extract has anticancer activity against colorectal cancer cell line HT-29 with IC50 87 µg/ml, and could increase caspase 3, 8 and 9 activity levels in the cells (Hajiaghaalipour et al., 2015). Other research suggests that LSG inhibits tumor growth by stopping the S phase of cell growth and decreasing the appearance of oncogenes. like ß-catenin, c-Myc, K-Ras, and the anti-apoptotic proteins Bcl-xl and Bcl2 (Anwar et al., 2018). According to study by Shamekhi et al. (2020), HKY inhibits the expression of p-Akt1, Rel A, Bcl2, pro-caspase 3, and pro-caspase 9, while increasing the expression of BAX, cleaved caspase-3, and cleaved caspase-9 in colon cancer cell lines.

The results of bcl-2 gene expression in WiDr cells after treatment with 125, 62.5, and 31.25 ppm extract concentration showed that some natural compounds in extracts can affect bcl-2 gene expression in cells. Some of these compounds can either increase or decrease bcl-2 gene expression in specific cells. In this case, treating WiDr cells with extracts of varying concentrations can decrease the expression of the bcl-2 gene in these cells.

The representation of apoptotic marker genes like bax and bcl-2 is required for the regulation of cell death and the suppression of cell proliferation. More investigation is required to support this theory and establish the impact of red snail extract treatment on cancer cells. Red snail extract treatment have the ability to change the appearence of these genes in WiDr colon cancer cells. In vitro anticancer action in colon cancer cells reduced cell development and promoted apoptosis (Sari et al., 2018). Earlier research indicated that red snail meat ethanol extracts at doses of 100 and 200 mg/kg BW/day markedly decreased SGOT and SGPT levels in the white rats serum (Trisdiani et al., 2022). It is important to remember that the findings of these studies must be validated and confirmed through additional research, as other factors such as incubation time, cell state, and the nature of the extract may influence the findings of these studies. However, more research is needed to prove this hypothesis, such as mRNA and protein levels of expression of genes as well as cell function analysis to determine whether the red eye sea snail extract treatment can affect cells' ability to induce apoptosis. Furthermore, strict controls and a large enough sample size are required to ensure reliable results.

CONCLUSION

Treatment with red eye sea snail extract increased the expression of the bax gene while decreasing the expression of the bcl-2 gene in WiDr colon cancer cells. It can be concluded that red eye sea snail extract has a potential as alternative therapy on cancer by inducing apoptosis in WiDr colon cancer cells by controlling the expression of the genes bax and bcl2. This study provides preliminary evidence of the anti-cancer properties of red eye sea snail extract. However, more research is needed to determine the processes governing bax and bcl-2 gene expression, as well as to determine the safety and effectiveness of using red eye sea snail extract as an anti-cancer treatment.

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AUTHORS CONTRIBUTION

WJD, EH, SP, and SM designed the study. WJD performed and analysed the study under supervision of EH, SP and SM. The first draft of the manuscript was written by WJD and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest in this research.

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