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EFFECT OF BASIL LEAVES EXTRACT (*Ocimum sanctum* L.) ON BCL-2 PROTEIN EXPRESSION IN T47D BREAST CANCER CELLS

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ABSTRACT

Breast cancer is one of the most common types of cancer in women throughout the world. This disease is characterized by the uncontrolled growth of cells in breast tissue. One of the molecular pathways that plays an important role in the development of breast cancer is the apoptosis pathway with a protein that prevents apoptosis, namely the BCL-2 (B-cell lymphoma 2) protein. Basil leaves (Ocimum sanctum L.) are a plant that can be used as an alternative treatment for cancer because they contain secondary metabolite compounds that have anticancer activity. Therefore, BCL-2 is an important target in developing breast cancer therapy, and the use of natural ingredients such as basil leaves is the main choice for alternative cancer treatment with lower side effects. This study aims to determine the impact of basil leaf ethanol extract on BCL-2 protein expression in T47D breast cancer cells. The extract was obtained using the maceration method with 96% ethano solvent and to determine the effect of p53 and Bcl-2 expression, an immunocytochemical test was carried out. Bcl-2 expression is indicated by the binding between the Bcl-2 protein and the anti-Bcl-2 monoclonal antibody which is detected as a brown color in the cytoplasm and membrane of T47D cells. The results showed that basil leaf extract at a concentration of 103.36 µg/ml was able to inhibit Bcl-2 expression by 54,63 % and the positive control (doxorubicin) at a concentration of 10.13 µg/ml was able to inhibit Bcl-2 expression by 76.78%.

Keywords: basil leaf extract; bcl-2 protein; breast cancer; T47D Cells

BACKGROUND

Breast cancer is one of the most common and deadly types of cancer in the world, especially in women(Sun et al., 2017). Current breast cancer treatment involves various methods such as surgery, chemotherapy, radiation, and hormone therapy. However, this treatment is often accompanied by significant side effects and drug resistance, so there is a need for alternative or additional therapy that is more effective and safe(Ahmad, 2019). One alternative effort to treat cancer is the use of natural ingredients that have the potential to be developed as breast cancer treatments. One plant that can be designed as a chemopreventive agent is basil leaves (Ocimum sanctum L.) . The active compounds contained in basil plants include flavonoids, orientin, visenin, eugenol, and ursolic acid (Dhandayuthapani et al., 2015).

The ethanol extract of basil leaves can be used as a chemopreventive agent because it can induce apoptosis of lung cancer cells, and has a cytotoxic effect on colon and prostate cancer (Wong, 2011). Basil leaf ethanol extract can induce apoptosis in lung cancer cells (A549) via mitochondrial caspase with an IC50 value of 176 µg/mL (Ahmad, 2019), and effectively induces apoptosis in prostate cancer cells (LNCaP) with an IC50 value of 116.18 µg/mL

through activation of caspase-9 and caspase-3 which can ultimately cause DNA fragmentation and cell death (Dhandayuthapani et al., 2015). Basil leaf essential oil has a cytotoxic IC50 value of 60 μg/mL against MCF-7 cells (Tamil Selvi et al., 2015). The ethanol extract of basil herb also has cytotoxic activity on WiDr colon cancer cells with an IC50 value of 85 μg/mL(Niture et al., 2006).

The Bcl-2 protein (B-cell lymphoma 2) is a protein that plays an important role in the regulation of apoptosis or programmed cell death. Overexpression of Bcl-2 protein is often associated with cancer cell resistance to chemotherapy and increased cancer cell survival. Mutations of the Bcl-2 gene can cause increased expression that can suppress the normal function of proapoptotic proteins. If this occurs in this protein, it can cause downregulation, so cells lose the ability to regulate apoptosis which can trigger cancer (Kapoor et al., 2020). Therefore, decreasing Bcl-2 expression may be an effective strategy in cancer treatment.

This study aims to determine the impact of basil leaf ethanol extract on BCL-2 protein expression in T47D breast cancer cells. The extract was obtained using the maceration method with 96% ethano solvent and to determine the effect of p53 and Bcl-2 expression, an immunocytochemical test was carried out.

METHODS

This research is a laboratory experimental research. Research was carried out to observe Bcl-2 activation using immunocytochemical methods. Materials used T47D cell line, p53 and Bcl-l2 genes, RPMI 1640 media (Gibco), sodium bicarbonate, HEPES (Sigma), Fetal Bovine Serum (FBS), penicillin-streptomycin 1% v/v (Gibco), DMSO (Dimethyl Sulfoxide), Trypsin 0.5% MTT 5mg/ml, PBS solution, Function 0.5% MTT 5 mg/ml, in PBS, cell washing media, PBS (Phosphate Buffered Saline) solution and Equipment for immunocytochemical tests is a nitrogen tank liquid, centrifuge, autoclave, Sigma 3K12 centrifuge (B, Braun Biotech International), Laminar Air Flow class II (Labconco), ELISA reader (SLT 240 ATC), Nebauer Haemocytometer (BDH Merck), sterile conical tube, 96-well microplate (Biologix), ultraviolet lamp, electric balance, 20-200 μL and 200-2000 μL micropipettes (pipetman).

Making extracts begins with collecting materials. A total of 5 kg of leaves were collected and then washed with running water to remove any dirt that was still attached, then drained and cut crosswise. Drying was carried out in the oven at 40°C. The extract was made using ethanol solvent using the maceration method, the extraction process was carried out for 5 days. The filtrate is then evaporated using an evaporator (temperature maintained at 400-500C) until a thick extract is obtained(Pertiwi et al., 2020).

The test used for BCL-2 gene expression was using the immunocytochemical method. A cell culture of 5x104 cells/well was transferred into a 24-well plate filled with a cover slip, then the cells were incubated overnight at 37^{o}C in a 5% CO2 incubator. After the cells recovered, they were treated with the extract and incubated again for 15 hours. Cells were washed with PBS then cold methanol was added and incubated in a -4oC freezer for 10 minutes. The cells that had been fixed were then washed with distilled water 2 times and then incubated in hydrogen peroxidase solution for 10 minutes. Next, the cells were dripped with prediluted blocking serum and incubated for 10 minutes then dripped with anti-p53 primary monoclonal antibodies. The preparations were incubated in biotin for 10 minutes and washed with PBS. After washing with PBS, cells were dropped with a secondary antibody (biotinylated universal secondary antibody) and incubated again for 10 minutes. The preparations were then soaked in Mayer-Haematoxylin solution for 3-4 minutes to counterstain and washed with distilled water. The cover slip is then removed and dipped in xylol, then dipped in

alcohol. Once dry, the cover slip is placed on a glass object and dripped with glue (mounting media). The cover slip was covered with a slide and then observed using a light microscope(Rahmawati, 2023). Data analysis with ImageJ software was carried out to quantitatively calculate Bcl-2 protein expression by calculating the number of threshold pixels (area) and the percentage of threshold pixels (% area).

RESULTS

Maceration with 96% ethanol filter produced a yield of 20.45%. The results of identifying compound groups using color reactions from basil leaf extract show that the extract contains flavonoids, alkaloids, saponins, polyphenols and triterpenoids.

Immunocytochemistry is a method used to detect the expression of a specific protein or antigen in cells by using specific antibodies that will bind to the protein or antigen. Tests were carried out on four treatment groups, namely the group without treatment (cell control), the group given basil leaf extract, and each with a concentration of ½ IC50 (25.84 µg/ml) IC50 (51.68 µg/ml) 2IC50. (103.36 µg/ml). Bcl-2 expression was indicated by the presence of binding between the protein and the anti-Bcl-2 monoclonal antibody which was detected in the form of a brown color in the cytoplasm and membrane of T47D cells. The results of immunocytochemical staining of T47D cells to see BCl2 expression can be seen in Figure 1

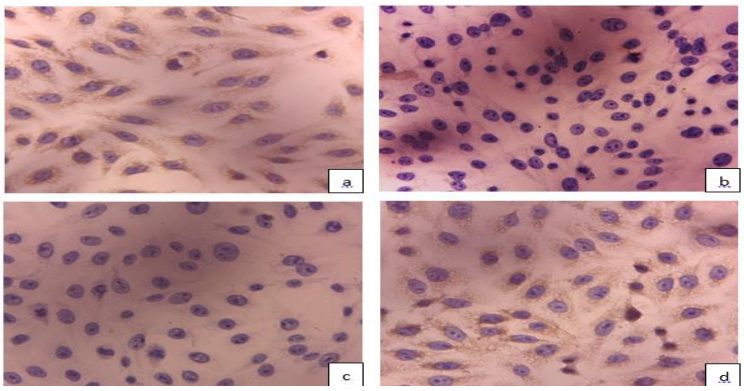

Figure 1. Results of observations of Bcl-2 gene expression. a) concentration of basil leaf extract 51.68 µg/ml, (b) concentration 103,36, (c) positive control, (d) Control cells without treatment. Observation under a light microscope with 400x magnification

The average value of the percentage of T47D cancer cells that express p53 and Bcl-2 after immunocytochemical staining for all treatment groups can be seen in Table 1.

Table 1. Results of percent expression of the Bcl-2 genes

Treatmen	**Concentration (µg/ml)**	expression of the Bcl-2 **(%) ± SD**
Extract	25,84 (½ IC_{50})	19,15 ± 0,55
	51,68 (IC_{50})	29,08 ± 0,90
	103,36 ($2IC_{50}$)	54,63 ± 0,55
Positif control	10,13 (IC_{50})	76,77 ± 0,95
Cell control	0	24,07 ± 0,97

DISCUSSION

The basil leaves that will be used for research are taken and collected from basil gardens in Ungaran, Semarang Regency, Central Java Province. Before the research was carried out, the basil leaf extract was identified to ensure the presence of flavonoids, polyphenols, saponins, alkaloids, and triterpenoids/steroids. The results of identifying compound groups using color

reactions from basil leaf extract show that the extract contains flavonoids, alkaloids, saponins, polyphenols, and triterpenoids.

This test uses T47D breast cancer cells. T47D cells are a model of breast cancer cells that are not yet resistant to the chemotherapy agent doxorubicin but are known to have a mutated p53 gene (Coutsouvelis et al., 2020). Testing in this way is better than in vivo testing because it is more economical, and safer and the testing time is relatively shorter(Kapoor et al., 2020).

Immunocytochemistry is a method used to detect the expression of a specific protein or antigen in cells by using specific antibodies that will bind to the protein or antigen. Administration of n-hexane extract and fraction can also inhibit the expression of Bcl-2(Kapoor et al., 2020). Cancer cells occur due to gene mutations, one of which is the Bcl-2 mutation. Mutations of the Bcl-2 gene can cause increased expression that can suppress the normal function of proapoptotic proteins. If this occurs in this protein, it can cause downregulation, so cells lose the ability to regulate apoptosis which can trigger cancer . It can be seen from the average percentage of expression of Bcl-2 that the greater the concentration of extract or n-hexane fraction given, the more it inhibits Bcl-2 expression. Bcl-2 expression is indicated by the presence of binding between the Bcl-2 protein and the anti-Bcl-2 monoclonal antibody which is detected as a brown color in the cytoplasm and membrane of T47D cells. The Bcl-2 protein is antiapoptotic, this protein plays a role in regulating apoptosis through regulating the release of Cyt c (cytochrome). The content of alkaloid compounds, through a mechanism involving ROS production, inhibits the release of mitochondrial cytochrome c resulting in a decrease in the level of the anti-apoptotic Bcl-2 protein(Qin et al., 2019). The results showed that basil leaf extract (Ocimum sanctum L.) was able to inhibit Bcl-2 expression.

CONCLUSION

The results showed that basil leaf extract at a concentration of 103.36 µg/ml was able to inhibit Bcl-2 expression by 54,63 % and the positive control (doxorubicin) at a concentration of 10.13 µg/ml was able to inhibit Bcl-2 expression by 76.78%.

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