Extract of Bitter Melon (Momordica Charantia L.) as a Cytotoxic and Anti Proliferaton Agent for Cells WiDr (Colon Cancer)

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Abstract. Colon cancer is one of the types of cancer that gives a high mortality rate. Many people with colon cancer do not realize they have colon cancer because the initial spread does not show severe symptoms. Colon cancer occurs due to cell abnormalities caused by DNA mutations. To reduce the death rate caused by cancer, there have been many attempts to find effective treatments, both modern and traditional. Modern treatments such as surgery and chemotherapy still often have adverse effects on sufferers. Therefore new efforts are made to find alternative treatments, one of which is the traditional way of exploring natural ingredients and utilizing secondary metabolites produced by plants. Pare (Momordica charantia L.) is an anticancer candidate characterized by the presence of cytotoxic saponins momordicosides, flavonoids, and alkaloids as inhibitors of cell development processes. This study's objectives were (1) To determine the cytotoxic effect of bitter melon (M. charantia L.) extract on reducing the number of colon cancer cells (WiDr), (2) To determine the effect of the proliferation of bitter melon (M. charantia L.) extract on reducing the number of colon cancer cells (WiDr). The results obtained for the cytotoxic test, namely the IC50 value of 111 µg / ml, were said to have quite toxic properties and were able to have anticancer activity. The proliferation test showed that EBP inhibited the proliferation rate at the 24-hour incubation period and had time to increase at a concentration of 13,875 µg / ml with values above 400 hours.

Keywords: Colon Cancer, Cytotoxic, Momordica charantia L., Proliferation.

1 Introduction

Cancer, also known as a malignant neoplasm, is a disease characterized by cell cycle abnormalities that cause cells' ability to grow out of control [1]. Colon (colorectal) cancer is one of the most common causes of death in the world, with America an estimated incidence of 75,610 cases in men and 64,640 cases in women with an overall average of about 80,000 deaths per year [2] while for Indonesia, colon cancer includes ten primary cancers are common [3].
Colon cancer, which grows on the surface of the colon (intestine) or rectum (anus), which is part of the large intestine in the digestive system, is also called the gastrointestinal tract, which functions as a producer of energy for the body and removes waste products that are not useful [4]. Today, medical practitioners generally have three ways of treating cancer, namely surgery, radiation, and chemotherapy [5].

Surgery is an invasive treatment procedure through incisions to open or reveal parts of the body that will help generally experience a high increase in cancer cells that have not metastasized (spread), the effects of surgery failure can cause cancer to spread to other body tissues and worsen the condition. Radiotherapy, which uses radioactivity to destroy tumor cells. The advantage is that it only causes minor damage to the surrounding normal tissue. The types of radiation rays commonly used are gamma rays (γ) and X-rays [6].

Chemotherapy is a treatment effort to kill cancer cells by giving synthetic chemotherapy drugs, unlike surgery or radiation locally, chemotherapy is spread throughout the body because it is a systemic therapy, which means the drugs given spread throughout the body. So that the resulting effect will make sufferers experience anemia, thrombocytopenia, leucopenia, nausea, vomiting, alopecia, stomatitis, allergies, pain and tissue necrosis [7].

Bitter melon has a bitter taste caused by the content of momordicosides of the triterpene glucoside group or kukurbitasin which are very patent antiproliferative and anti-differentiation properties. Efforts to develop alternative preparations in traditional medicine that can replace synthetic chemotherapy drugs and are relatively more effective in increasing body immunity. Traditional medicine that is often used comes from natural ingredients, namely plants, by knowing secondary metabolite compounds' content.

bitter melon contains saponins and is cytotoxic. Cytotoxics are substances or compounds that can damage cancer cells. Flavonoids inhibit a number of cell development processes in the body through inhibition of a number of enzymatic reactions as well as potential anti-cancer drugs. The United States NCI (National Cancer Institute) states that an extract or compound can be said to be potential as an anticancer agent if it has an IC value of <50 µg / ml and if an extract or compound has an IC value of > 140 µg / ml then the extract or compound is said to be not. It is toxic and has no anticancer activity [8]. Proliferation inhibition activity can occur due to alkaloid and flavonoid compounds in the bitter melon extract that can stimulate enzymes to inhibit the cell cycle, as an antiproliferation and angiogenesis of cancer cells [9]. The mechanism for inhibiting proliferation occurs probably because cells die. This cell death can go through the cell cycle mechanism to stop (arrest) by stopping the cell cycle, so the cells cannot reproduce themselves.

2 Method

2.1. Material

The material used in this research was bitter melon, 96% ethanol, cell culture for cytotoxic and proliferation test: culture media in the form of Roswell Park Memorial Institute (RPMI), phosphate buffer saline (PBS), solvent methanol, dimethyl sulfoxide (DMSO), propidium iodide, trypsin EDTA, microtetrazolium (MTT), sodium dodecyl sulfate (SDS) 10%, ethidium bromide-acridine orange.

2.2. Equipment

Include a set of extraction tools in the form of a cutting knife, plastic tub/trough, oven, mortar, Buchner, funnel and filter paper, rotatory evaporator. The cytotoxic and proliferation
test kits for colon cancer cells are liquid nitrogen tank, water bath, laminar airflow, refrigerator, Eppendorf tube, centrifuge tube, centrifugation, micropipette 10, 20, 200, and 1000µL, small test tube, six and 96-well plate, conical tube, yellow tip and blue tip, Elisa reader, vortex, coverslip, hemocytometer, CO2 incubator, the waste container for used media for phosphate buffer saline (PBS), tissue, aluminum foil, fluorescence microscope.

2.3. Research Implementation
This research consists of several stages, namely 1). Sample preparation ; 2). WiDr cell cytotoxic test (Microtetrazolium method); and 3). WiDr cell proliferation test (Doubling time Methode). This research stage is presented in figure 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Indicator</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Preparation</td>
<td>Has a like pasta texture.</td>
<td>I am lost of EBP pasta for stock.</td>
</tr>
<tr>
<td>Cytotoxic Test</td>
<td>WiDr cells are unable to survive due to the addition of the toxic compound EBP.</td>
<td>The decrease in the number of WiDr cells.</td>
</tr>
<tr>
<td>Proliferation Test</td>
<td>WiDr cells experience slow growth (inhibited) by compounds from EBP.</td>
<td>The decrease in the number of WiDr cells.</td>
</tr>
</tbody>
</table>

Figure 1. Research Implementation

3 Result and Discussion

The results obtained for cytotoxic were the highest average absorbance of living cells after being given EBP at a sample concentration of 50µg/ml with a value of 101,0737%. The lowest was at a concentration of 275µg/ml, with a value of 0,572656%. With an increase in EBP concentration, it can have a cytotoxic effect on WiDr cells so that the number of WiDr cells decreases; however, based on the data in Table 1, the average percentage of living cells gives varied results, even at the highest concentration of 300µg/ml EBP gives the value of % living cells of 2,791696 which is lower than the percentage of living cells at the concentration below.

It is assumed that too high a concentration is not too good and can result in cells having higher adaptability. To determine how toxic a substance or extract is, it is necessary to calculate the IC50 value. IC50 can be seen directly from the number 50 on the percentage results of the ability to live cells. The number 50% of living cells fall into the concentration range between 125µg/ml to 100µg/ml. The IC50 value obtained was 111 µg/ml. These results are used for the proliferation test.

Table 1. Mean Percent Absorbance of Live Cells

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Absorbance EBP</th>
<th>Mean % Live Cell</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
<td>P3</td>
</tr>
<tr>
<td>300</td>
<td>0,143</td>
<td>0,137</td>
<td>0,140</td>
</tr>
<tr>
<td>275</td>
<td>0,137</td>
<td>0,126</td>
<td>0,126</td>
</tr>
</tbody>
</table>
Based on the data above, EBP has an IC value of < 150 µg / ml, and it can be said that EBP is categorized as quite toxic and has anticancer activity. This is evidenced by the research [10] that the IC50 value < 150 µg / ml is categorized as quite toxic and has a positive correlation as an anticancer agent. The United States NCI (National Cancer Institute) states that an extract or compound can be said to be potential as an anticancer agent if it has an IC value of < 50 µg / ml and if an extract or compound has an IC value of > 140 µg / ml then the extract or compound is said to be not. It is toxic and has no anticancer activity [8]. The chemical content of unripe bitter melon fruit, which has medicinal properties, is saponins, flavonoids, alkaloids, polyphenols [11], and cucurbitacin glycosides, charantin, butyric acid, steroid compounds, monocyclic alcohol, and some triterpenoid compounds [12]. This is following the latest research conducted by [13], which states that the ethyl acetate fraction of 70% ethanol extract from bitter melon has high toxicity properties to HeLa cells with an incubation period carried out within 24 hours and 48 hours of the ethyl acetate fraction from ethanol extract 70% incubation 24 and 48 hours amounted to 34,9221 and 22,1871 µg/ml.

| 250 | 0,130 | 0,137 | 0,123 | 0,644238 | 0,007000 |
| 225 | 0,302 | 0,126 | 0,136 | 13,09950 | 0,098853 |
| 200 | 0,354 | 0,147 | 0,146 | 19,04080 | 0,119801 |
| 175 | 0,207 | 0,209 | 0,201 | 16,89334 | 0,004163 |
| 150 | 0,264 | 0,273 | 0,31 | 33,35719 | 0,024379 |
| 125 | 0,358 | 0,362 | 0,353 | 49,53472 | 0,004509 |
| 100 | 0,460 | 0,445 | 0,465 | 70,79456 | 0,010408 |
| 75  | 0,545 | 0,579 | 0,558 | 93,12813 | 0,017156 |
| 50  | 0,614 | 0,611 | 0,568 | 101,0737 | 0,025736 |
| 25  | 0,578 | 0,592 | 0,575 | 97,63780 | 0,009074 |

The results of the proliferation test using the Doubling Time method can be seen in Figure 2. It proved that the adequate concentration time to inhibit cell growth is in the 24-hour incubation period. This is following that at 24 hours, and there is a decrease in the number of living cells. The highest doubling time value, namely at a concentration of 1 / 8IC50 or 13,875 µg/ml, had a length of time for cells to divide into two times the initial time at each incubation.
time of 400 hours. Meanwhile, the concentration of 2IC$_{50}$ or 222 µg/ml has a shorter doubling time than other concentrations, which is at 100 hours. This can be caused by cell death. This cell death can go through the cell cycle mechanism to stop (arrest) by stopping the cell cycle, so the cell cannot reproduce itself.

Proliferation inhibition activity can occur due to the presence of alkaloid and flavonoid class compounds contained in bitter melon fruit extract, as stated by [14] that the positive bitter melon contains secondary metabolite compounds, namely flavonoids. Flavonoids can stimulate enzymes to inhibit the cell cycle as an antiproliferation and angiogenesis of cancer cells [7]. The mechanism for inhibiting proliferation occurs probably because cells die. This cell death can go through the cell cycle mechanism to stop (arrest) by stopping the cell cycle, so the cell cannot reproduce itself. According to [15], proliferation inhibition can also occur through the formation of DNA fragmentation, decreased Bcl mRNA expression, and increased Bax mRNA expression.

4 Conclusion

Conclusions that can be drawn from the research conducted bitter melon fruit are: (1) the cytotoxic effect of bitter melon extract can reduce the number of colon cancer cells (WiDr). Bitter melon are toxic to colon cancer cells WiDr, for the cytotoxic test, namely the IC$_{50}$ value of 111 µg / ml, was said to have quite toxic properties and were able to have anticancer activity. (2) The proliferative effect of bitter melon extract can inhibit proliferative activity and reduce the number of colon cancer cells (WiDr). The bitter melon fruit extract can also reduce the growth of colon cancer cells WiDr by reducing the rate of cell proliferation. The proliferation test showed that EBP inhibited the proliferation rate at the 24-hour incubation period and had time to increase at a concentration of 13,875 µg / ml with values above 400 hours.

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References


