THE INHIBITION OF POLYSOPRENOID FROM NYPHA FRUTICANS LEAVES ON CYCLOOXYGENASE 2 EXPRESSION OF WIDR COLON CANCER CELLS

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ABSTRACT

Objective: The objective of the study was to investigate the inhibitory activity of polysoprenoids from Nypa fruticans leaves on the expression of cyclooxygenase 2 (COX-2) against colon cancer cells.

Methods: Anticancer activity performed was tested by dimethylthiazol-2(5)-tetrazolium bromide method on colon cancer cell WIDr. The expression of COX-2 was observed by the immunocytochemistry method.

Result: Polysoprenoids from N. fruticans leaves exhibit anticancer activity on WIDr cells through inhibition of COX-2 expression with IC50 180.186 ± 7.16 μg/mL.

Conclusions: This study showed that polysoprenoids from N. fruticans leaves promise chemopreventive agents for colon cancer through COX-2 inhibition.

Keywords: Cytotoxic, Polysoprenoids, Nypa fruticans, Cyclooxygenase-2.

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INTRODUCTION

Cancer is one of the primary causes of death worldwide. Lung, liver, stomach, colon, and breast cancers are the biggest causes of cancer deaths annually [1]. Colon cancer is the third most frequently diagnosed cancer causing health problems [2] as the fourth leading cause of cancer death worldwide. There is an increasing number of colon cancer patients each year. The incidence of colon cancer mainly occurs because it is influenced by socioeconomic level, behavior, and lifestyle [2]. Low physical activity and high fat consumption cause easy absorption of carcinogenic compounds in the body and slow the transport time to the intestine which can lead to increased risk of colon cancer [2]. Increased incidence of colon cancer or colon cancer mortality was found in countries with low Human Development Index (HDI) levels, especially in Eastern Europe, Asia, and South America. On the contrary, the incidence of colon cancer and death from colon cancer has stabilized or decreased in a number of high HDI, such as the United States, Australia, New Zealand, and some countries in Western Europe [4].

Arachidonic acid metabolism is thought to play a significant role in the occurrence of carcinogenesis [5]. This metabolic pathway is associated with the formation of prostanooids. Prostanooids belong to the subclass of eicosanoids. They are produced by the conversion of arachidonic acid into prostaglandins [6]. Cyclooxygenase (COX) is a critical enzyme in the conversion of arachidonic acid into prostaglandin [7].

The rapid development of the pharmaceutical industry in creating synthetic drugs currently aims to prevent and treat cancer, but most are toxic. Treatment technologies such as surgery, radiation, and chemotherapy are performed by administering anticancer drugs. This type of therapy makes patients experience nausea, vomiting, and hair loss [8]. Recommended companion therapy is the use of compounds that can reduce the effects of growth factors that may stimulate the rapid growth of cancer. Therapies aimed at enhancing the immune response to cancer also began to be widely tested in clinical studies. One component that has a high chance of being used in cancer therapy is medicinal plants [9]. Medicinal plants can be used for various functions and one of them as an inhibitor of COX-2 protein [10].

Mangrove has activity as a medicinal plant, and only a few have been explored [11]. Mangrove is famous for producing secondary metabolite compounds mainly from isoprenoid compound groups. The polysoprenoids compound consists of two families, namely polypropenol and dolichol, polypropenol is known to have some pharmacological activity such as anticancer [12], antidiabetic [13], anti-infection, and antiviral activity [14].

The distribution and diversity of polysoprenoids compound in mangrove forests of Iriomote Island, Japan and North Sumatra, Indonesia have been reported by Basyuni et al. [15,16]. The promising mangrove species that potentially exhibit anticancer activity are Nypa fruticans [17]. However, the cytotoxic activity of polysoprenoids from N. fruticans leaves on colon cancer cells with COX-2 as a target molecule is unclear. Therefore, this study aims to examine the cytotoxic activity of polysoprenoids from N. fruticans leaves on high-frequency colon cancer cells expressing COX-2.

METHODS

Plants and isolation of polysoprenoids

The sample used in this research is the leaves of N. fruticans obtained from the Lubuk Kertang Village, North Sumatra. An extract obtained from the leaves of N. fruticans was performed as described previously [15,16,18]. The leaves of N. fruticans were dried for 2 days at 60°C and then crushed to a powder. The leaves powder was extracted with a mixture of chloroform/methanol (2:1, v/v) for 48 h, then filtered and the remaining was a lipid extract in chloroform. The lipid extract in the chloroform of the leaves was precipitated at 65°C.
Cell culture and conditions

The W.Dr cell line used in this study is a collection of the Parasite Laboratory of Gadjah Mada University, Jogjakarta, Indonesia. W.Dr were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) with 15% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, were purchased from Gibco (Carlsbad, CA, USA). Cell culture was performed in incubators at 37°C, 5% CO₂.

Cytotoxic test

The cytotoxic activity of polysipeptides from N. fruticans leaves was assessed by the method of MTT test [19] using a tetrazolium microculture (MTT) method with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The MTT solution was purchased from Sigma-Aldrich (St. Louis, MO, USA), penicillin and streptomycin, and Sigma-Aldrich (St. Louis, MO, USA) antibiotics were purchased from Gibco (Carlsbad, CA, USA). Phosphate buffer saline (PBS) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and incubated in an incubator at 37°C, 5% CO₂.

Percent of inhibition is calculated based on the following equation:

\[
\text{%Inhibition} = \frac{\text{Medium absorbance} - \text{Untreated cell absorbance}}{\text{Medium absorbance}} \times 100
\]

The correlation between the percentage of inhibition and the concentration of polysipeptides from N. fruticans leaves was plotted, and IC₅₀ was calculated through their interpolation through regression equation. IC₅₀ was the concentration of polysipeptides from N. fruticans leaves inhibiting the growth of 50% treated cells and cell morphology becomes abnormal.

Observation of COX-2 protein expression with immuno cytochemistry

Analysis of inhibition of COX-3 protein expression using immunocytochemistry methods was performed as described previously [20]. The W.Dr cell lines were seeded on 24-wells plates first, included coverslip on each well. Cells were seeded with a density of 5 x 10⁴ cells/well, incubated for 24 h at 37°C with 5% CO₂. Furthermore, the polysipeptides from N. fruticans leaves were incubated in various concentrations (0, 10, 50, 100 μg/ml). The cells were fixed with 4% paraformaldehyde for 30 min, washed 3 times with PBS, and then with 0.1% Triton X-100. After washing twice with PBS, each well was treated with blocking solution for 30 min. The cells were incubated with COX-2 monoclonal antibody (1:100) overnight at 4°C. Then, the cells were washed with PBS, and then incubated with secondary antibody for 1 h at room temperature. The cells were then washed with PBS, and mounted with Vectashield Mounting Medium with DAPI. The images were captured using a fluorescence microscope.

Results

This study examined the cytotoxic effects of polysipeptides from N. fruticans leaves on colon cancer cell W.Dr with an inhibitory observation on COX-2 protein. Cytotoxic effects were measured with MTT test, then measured the absorbance of formazan complex at a wavelength of 595 nm equivalent to the number of living cells. The results of the viability of colon cancer cells after administration of polysipeptides from N. fruticans leaves in various concentrations are shown in Fig. 1. Polyseptides from N. fruticans leaves (R²=0.9158) showed cytotoxic activity in colon cancer cells W.Dr depending on concentration. The IC₅₀ value of polysipeptides from N. fruticans leaves was 180.1867±16 μg/ml.

The linear regression equation can be seen in the comparison graph with live cell percentage. From the results obtained, there is a decrease in the number of living cells based on the increased concentration given. Concentration 250 μg/ml has the best inhibition of cancer cell W.Dr with a small percent of living cells. The polysipeptides from N. fruticans leaves obtained linear regression equation Y=-0.4891x+139.94. From the derived linear regression can be calculated the IC₅₀ value. The value of IC₅₀ obtained from polyseptides N. fruticans leaves is 180.1864 μg/ml from the calculation of concentration to live cells percentage.

Effect of polysipeptides from N. fruticans on COX-2 expression suppression

In this study, observation of protein suppression test of COX-2 has been done by immunocytochemistry which then analyzed qualitatively and quantitatively. From the qualitative analysis, it shows that with increasing test extract levels, the positive COX-2 expressing cells are less, indicating that there has been a decrease in COX-2 expression in W.Dr cells. Immunocytochemistry coloring results can be seen in Fig. 2. Furthermore, quantitative analysis was done to find out the percentage emphasis of COX-2 protein expression by polysipeptides from N. fruticans leaves. Quantitative suppression of COX-2 expression is done by calculating the percentage of COX-2 expression. The results of the calculations can be seen in Table 1. In Table 1, it can be seen that the increasing concentration of polysipeptides from N. fruticans leaves of 90 μg/ml and 180 μg/ml and gives a decrease of COX-2 expression pressure 36.83±2.01% and 16.42±2.86%, respectively. While the average control of COX-2 suppression was 68.13±3.07. It has been shown that administration of Polyseptides from N. fruticans leaves 90 μg/ml inhibits COX-2 protein expression. Similarly, administration of

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<td>Untreated</td>
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<td>90 μg/ml</td>
<td>Polysipeptides from N. fruticans leaves 36.83±2.01*</td>
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<tr>
<td>180 μg/ml</td>
<td>Polysipeptides from N. fruticans leaves 16.42±2.86*</td>
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*Referred to the significant difference to untreated cells (p<0.05).
Fig. 1: Effect treatment of polysperonoids from Nypa fruticans leaves on colon cancer cell lines WiDr.

Fig. 2: Immunocytochemistry results of cyclooxygenase-2 (COX-2) on WiDr cells qualitatively. (A) The negative control, the group untreated with COX-2 specific antibody coloring. Visible cells are brown, showing a positive result against COX-2. (B). Treatment of polysperonoids from Nypa fruticans leaves 50 μg/mL. (C). Treatment of polysperonoids from Nypa fruticans leaves 180 μg/mL. (D). The yellow arrows showed the positive result of COX-2, the red arrow shows the negative result of COX-2. Observations under a light microscope with the magnification of ×40.

Polysperonoids from N. fruticans leaves 180 μg/mL shows that particle cells are clearer than controls. Furthermore, the percentage of COX-2 expression suppression data in each treatment and control group was statistically analyzed using way-one ANOVA parametric statistical analysis. Followed by Duncan’s test. Statistical analysis showed that COX-2 protein expression suppression in various treatment and control groups gave significant difference (p<0.05). The increase in the level of the given test extract was able to provide significantly increased percentage COX-2 expression when compared with the control.

DISCUSSION

This study evaluated the antitumor activity of polysperonoids from N. fruticans leaves on colon cancer cells WiDr. Polysperonoids from N. fruticans leaves tested against colon cancer cell WiDr have IC50 value 180.16±7.16 μg/mL. The IC50 value in ranking from 100 to 300 is considered as a weak antitumor activity, whereas the IC50 value over than 300 is regarded as inactive compounds [21]. This suggests that polysperonoids from N. fruticans leaves have a potent cytotoxic effect on WiDr cells.

This study evaluated the antitumor activity of polysperonoids from N. fruticans leaves on colon cancer cells WiDr. The inhibition of COX-2 expression. Many studies have been conducted both in vitro and in vivo on the activity of COX-2 inhibitors as cancer therapy [22,23]. The inhibition of COX-2 protein is an effective strategy to screen for chemopreventive agents in colon cancer [24]. Our results conclude that polysperonoids from N. fruticans leaves have antitumor activity in WiDr cells by inhibiting COX-2 expression. The results of this study are consistent with previous studies that polysperonoids exhibit antitumor activity on colon cancer [25]. N. fruticans leaves have been reported to contain polysperonoids, with majority diterolich compounds, no polyphenol detected [16]. Secondary metabolite compounds mainly from the group of isoprenoid also known as terpenoids, affect antitumor activity that has been tested through the ability to block nuclear factor-kappa B, induce apoptosis, activate transcription, and angiogenesis. Although the mechanism is unclear, terpenoids can be useful in the treatment of various types of cancer [27]. COX-2 inhibitory activity by polysperonoids from N. fruticans leaves with immunocytochemistry. The observed expression of COX-2 (Fig. 2) showed COX-2 expression due to polysperonoids treatment from N. fruticans leaves decreased compared to control. The inhibitory activity of COX-2 expression by polysperonoids from N. fruticans leaves may be due to inhibition of nuclear factor-kappa B. Terpenoids contained in N. fruticans leaves inhibits NF-kB and IκB-α [28]. This present results in a decrease of COX-2 expression.

COX-2 is an enzyme responsible for inflammatory response and prostaglandin production [29] and high expression in tumor cells [30]. Prostaglandins are reported to play a role in vascular endothelial growth factor regulation and induce angiogenesis in tumor cells [31]. Thus, it is suspected that the activity polysperonoids from N. fruticans leave as antitumor is mediated by COX-2 inhibition as one of its mechanisms. The active compounds are primarily responsible for all these effects have not been further investigated, but it is suspected that the active compound was terpenoids. Terpenoids play a role in the regulation of the COX-2 expression [32]. Previous studies have shown similar relationships between terpenoids and anticancer effects [33,34]. The triterpenes and sterols were reported to exhibit antioxidant and antitumor properties [35]. The COX-2 expression assessment provides information on the growth and determines the treatment modalities. Treatment using COX-2 inhibitors can be done when in part encountered excessive COX-2 expression. The angiogenesis process as an indicator of the aggressiveness of solid tumors is also essential in scores on the growth of colon cancer. Further research is needed to investigate the effects of polysperonoids from N. fruticans leaves in suppressing COX-2 expression. This experiment is expected to enrich the scientific evidence of polysperonoids activity from N. fruticans leaves as a chemopreventive agent, specifically to colon cancer.

CONCLUSIONS

Polysperonoids from N. fruticans leaves promise as a chemopreventive agent in colon cancer. Our data showed that polysperonoids from N. fruticans leaves inhibit expression of COX-2. Therefore, inhibition of COX-2 is one of the targeted therapy options developed for the treatment and prevention of cancer. Studies relating to the discovery of COX-2 inhibitor compounds still need to be developed to achieve maximum inhibitory effect but with minimal skill effect.

ACKNOWLEDGMENT

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AUTHOR’S CONTRIBUTION

Collection of N. fruticans leaves: DPS, MB, and BW; performed the experiments and analyzed the data. DPS and BW draft preparation. DPS, paper writing: DPS, MB, BW, and PAZIUL. All of the authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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