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Anticancer Potential of Cinnamon Bark Extract (Cinnamomum burmanii) with Cisplatin Combination against P-glycoprotein and Apoptotic Influx Biomarkers

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Abstract

AIM: The objective of the study was to demonstrate the effectiveness of cinnamon bark extract combines with cisplatin in reducing efflux and increasing influx in SKOV3 ovarian cancer cell cultures by measuring the expression of p-glycoprotein, CTR1, and the annexin V.

METHODS: This research is an experimental study using SKOV3 ovarian cancer cells stored in the SCTE IMERI FKUI Laboratory, carried out in the Stem Cells and Tissues Engineering Research Cluster laboratory. The cells were then harvested by adding trypsin-EDTA to the culture as much as 1 ml and rotated at 2000 rpm for 5 min. Then, the cells were added with antibodies and dissolved with a stain buffer solution and, read on a flow cytometry device. We used ethyl acetate extract from cinnamon bark against the SKOV3 cell line.We got IC50 of cinnamon bark extract from the MTS test. We tested the levels of IC50, $^{3/4}$IC50, $^{1/2}$IC50, and $^{1/4}$IC50 of cinnamon bark extract with a combination of IC50, $^{3/4}$IC50, $^{1/2}$IC50, and $^{1/4}$IC50 of cisplatin, against the viability of the SKOV3 cell line with a single cisplatin IC50 comparator. We also examined the levels of annexin V as a marker of apoptosis in the SKOV3 cell line to see if the cell cycle arrest induced by cinnamon bark extract could cause apoptosis of the SKOV3 cell line. We assessed the sample distribution using the Shapiro–Wilk test because of the sample size ≤50 samples. To assess the comparison of parameters (differences in p-glycoprotein and CTR-1 expression between treatment groups in normally distributed data), the test was used analysis of variance (ANOVA). ANOVA is a comparative test to analyze the difference in the mean (mean) of data from two or more variables in the same population. The Bonferroni test was used to analyze the same or different samples (equal and unequal) in each treatment.

RESULTS: This study found that the combination of IC50 cinnamon bark extract and IC50 cisplatin was able to lower p-glycoprotein levels higher with a lower mean value than the other treatment groups with $p < 0.001$. In the test group, the lowest p-glycoprotein expression was found in the combination 1 test group, namely, the 1 × IC50 combination. The value of the p-glycoprotein expression in the combination group 1 was 1.20%. As for CTR 1, the combination of IC50 cinnamon bark and IC50 cisplatin had the highest CTR1 levels among the three other treatment groups, with $p > 0.001$. In the test group, the highest CTR1 expression was found in the combination 1 test group, namely, the 1 × IC50 combination. The value of CTR1 expression in the combination group 1 was 12%.

CONCLUSION: Combining of cinnamon bark extract with cisplatin was shown to reduce efflux by decreasing p-glycoprotein expression and increasing influx by increasing CTR1 expression in SKOV3 ovarian cancer cell cultures.

Introduction

Ovarian cancer is the seventh most common malignancy in women, with more than 238,000 diagnosed cases and 151,000 deaths in 2012 [1]. Ovarian cancer is the fifth leading cause of cancer death among women in developed country [2]. In the United States, there were an estimated 22,280 new ovarian cancer cases and 14,240 deaths in 2016 [3]. The highest incidence (respectively, 10–12/100,000) is found in West Europe, North Europe, and North America where the lowest incidence (below 3/100,000) is located in China and Africa [4].

Ovarian cancer is treated with a combination of cytoreduction surgery and adjuvant chemotherapy. Adjuvant chemotherapy has progressed from single to the combination. Chemotherapy approaches now incorporate the addition of targeted therapies [5]. Cisplatin, cisplatinum, or cis-diamminedichloroplatinum (II) are one of the adjuvant chemotherapies drugs that are widely used as an anticancer because of its wide benefits in the treatment of various types of cancer [6]

but almost 70% of cisplatin-based chemotherapy has resistance in ovarian cancer cell [7]. Several mechanisms that play a role in platinum resistance are due to interference with the platinum influx process through the CTR-1 receptor, and an increase in efflux mediated by p-glycoprotein. Several studies have found that the combination of cisplatin chemotherapy with other drugs is very effective in overcoming not only drug toxicity but also in reducing chemotherapy resistance [8].

Nowadays, the use of phytopharmaceuticals is getting more and more attention because of its abundant availability in Indonesia, at affordable prices. Cinnamon is one of the phytopharmaceuticals that have been studied extensively and, in several studies, has been reported to have anti-cancer properties. Yang *et al*. reported that cinnamon bark essential oil has anticancer activity in head-and-neck squamous cell carcinoma by inhibiting the activity of EGFR-TK [9]. Dutta and Cahkraborty in their scientific review stated that cinnamon bark can increase the effectiveness of other anti-cancer, possibly through its effect on the CTR-1 receptor which helps the influx of platinum into cells [10]. This prompted us to further investigate the effect of the combination of cinnamon with cisplatin on SKOV3 cells, by assessing the biomarkers of influx (CTR-1) and efflux (p-glycoprotein) to see if cinnamon could increase the effectiveness of cisplatin in ovarian cancer treatment.

Materials and Methods

This research is an experimental study, using the ethyl acetate fraction of cinnamon bark on SKOV3 biological cell culture obtained from the SCTE IMERI Laboratory of FKUI carried out under research ethics as regulated in the Helsinki Declaration and obtained ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, University Indonesia – RSPUN Dr. Cipto Mangunkusumo Jakarta, with a research period from September 2020 to November 2021. These SKOV3 cell cultures are cisplatin-resistant cells, so we used doxorubicin in this study as a positive control, while in the negative control, we used a placebo. We then combined single cisplatin with cinnamon extract compared with single cisplatin and doxorubicin and tried to see their effect on the viability of SKOV3 cell cultures.

Cinnamon bark extract

The extract used in this study was *Cinnamomum burmannii*, another name for cinnamon bark, which came from the cinnamon tree forest plant in the hilly area of Gunung Raya subdistrict, Kerinci district, Jambi province. The bark of the tree is peeled from the trunk of the cinnamon tree which can be harvested at the age of 15−20 years. The bark of the tree is still fresh green, and mossy should be scraped before drying in the sun for 3 days. Dry skin will be rolled or folded. Before sorting the dried cinnamon, bark is stored at room temperature for 1 month so that the water content is no longer there. Sorting based on the thickness of the cinnamon bark, which is the thickest and the driest, is used as the sample of this study.

Cinnamon bark simplicia

Cinnamon bark collected, wet sorting is carried out to separate the dirt or foreign material, washed under running water, drained, and then spread on parchment paper until the water is absorbed then the material is weighed, then dried in a drying cabinet to dry, dry sorting to separate foreign objects such as unwanted plant parts and other impurities that are still present and left on the dried Simplicia, and then weighed and stored in a tightly closed plastic container protected from sunlight.

Cinnamon bark ethanol extract

A total of 500 g of Simplicia powder were put into a maceration container, then 10 parts of 96% ethanol were added to the powder, namely, 5000 ml or until the whole powder was submerged, stirred for 6 h periodically and then covered, and left for 18 h protected from light. Then filtered, the remaining residue is then soaked again using ethanol, or the same thing is done 3 times. The whole macerate was allowed to stand and then evaporated using a rotary evaporator at a temperature of 40°C to 50°C, until a thick extract was obtained.

Cinnamon bark extract fraction preparation

Fractions were made by liquid extraction (ECC) using n-hexane and ethyl acetate as solvents. A total of 10 g of ethanol extract were added to 40 ml of homogenized distilled water, put into a separating funnel, extracted with the first 50 ml of n-hexane, shaken, and allowed to stand until 2 layers were formed, namely, the n-hexane fraction and the water fraction (remaining). The n-hexane fraction was collected and fractionated until the n-hexane layer was clear. The water fraction (waste) was then extracted with the first 50 ml of ethyl acetate, shaken, and allowed to stand until two layers were formed, namely, the ethyl acetate fraction and the water fraction (residual). The ethyl acetate fraction was collected, and fractionation was carried out until the ethyl acetate layer was clear. The n-hexane, ethyl acetate, and water fraction (remaining) were evaporated using a rotary evaporator to obtain a thick extract [7].

Preparation of extract

Weighed 25 mg thick extract and dissolved with methanol up to 25 mL, obtained a solution with a concentration of 1000 ppm. Take 0.2 mL; 0.4 mL; 0.6 mL; 0.8 mL; and 1 mL of the 1000 ppm extract solution, then added 2 ml of DPPH solution (200 ppm concentration) at each concentration and added with methanol to the limit mark (10 mL volumetric flask), obtained concentrations of 20, 40, 60, 80, and 100 ppm. Incubated for 30 min then measured the absorbance using a UV–Vis spectrophotometer at a maximum wavelength of 515 nm.

Calculation of IC50 value

The calculation of the results of the DPPH trapping method is to calculate IC50, this value indicates that plant extracts can reduce as much as 50% of DPPH activity.

Preparation of cinnamon bark extract test solution

The extract was weighed as much as 12.5 mg was put into a 25 ml volumetric flask and was added using DMSO (dimethyl sulfoxide) to solve with a concentration of 500 g/ml. To obtain a solution with a concentration of 50, 75, 100, 150, 200, and 300 g/ml then the dilution was carried out, by pipetting 1 ml; 1.5 ml; 2 ml; 3 ml; 4 ml; and 6 ml. Each was put into a 10 ml volumetric flask and filled with DMSO until the marking line.

CTR-1 and p-glycoprotein expression analysis using flow cytometry

The treated cells were harvested by adding trypsin-EDTA into the culture container as much as 1 mL. Cells were then collected and rotated at 2000 rpm for 5 min. The supernatant was discarded, the cell pellet was then resuspended with 1x PBS solution. Cells were put into flow cytometry tubes and redissolved with stain buffer solution and rotated at a speed of 2100 rpm for 5 min. The supernatant was discarded, and the cell pellet was added with p-glycoprotein-FITC antibody and incubated for 15 min at room temperature and dark conditions. After incubation, the cells were then washed with a stain buffer solution, then rotated at a speed of 2100 rpm for 5 min. Cells were then added with 1 mL of Cytofix solution, then incubated at room temperature and dark conditions for 10 min. The cells were then rotated at a speed of 2100 rpm for 5 min, after centrifugation was completed, the supernatant was discarded, and the cell pellet was washed with 1 mL perm wash buffer solution, then the cells were vortexed and rotated at a speed of 2100 rpm for 5 min. The supernatant then discarded, and cytochrome C-PE and MCTR ATPase-APC-Cy7 antibodies were added to the cells. Cells were incubated for 20 min at room temperature and dark conditions. After incubation, the cells were then washed with 1 mL perm wash buffer solution and rotated at a speed of 2100 rpm for 5 min. The supernatant was discarded, and the cell pellet was resuspended or redissolved with a stain buffer solution. Cells are ready to be read on a flow cytometry device.

Data processing and analysis

Data processing and analysis were carried out using SPSS with a significance limit of $p < 0.05$. The data presented are in the form of mean standard deviation because the data are normally distributed. To prove that the data are normally distributed, the Shapiro–Wilk test is performed because of the number of samples ≤50. To assess the comparison of parameters (differences in CTR1 and p-glycoprotein expression) between treatment groups on normally distributed data, the test was used. Analysis of variance (ANOVA) is a comparative test to analyze the difference in the mean (mean) of data from two or more variables in the same population. Because the results obtained showed a significant difference, it was continued with the Bonferroni *post hoc* test. The Bonferroni test was used to analyze the same or different samples (equal and unequal) in each treatment. The Bonferroni test allows making comparisons between treatments, between treatments and treatment groups, or between treatment groups.

Results

In this study, the IC50 value of cinnamon was 68.73 ug/Ml and IC50 cisplatin was 117.5 uM.

Examination using flow cytometry

Cell-specific markers were examined using the BD CANTO II flow cytometry. The examination was carried out on the control group and the test group. In presenting the data below, the combination terms 1, 2, 3, and 4 will be used to facilitate reading in the table. Combination 1 is a combination of 1 × IC50 cinnamon bark extract and cisplatin. Combination 2 is a combination of × IC50 cinnamon bark extract and cisplatin. Combination 3 is a combination of × IC50 of cinnamon bark extract and cisplatin. Combination 4 is a combination of × IC50 cinnamon bark extract and cisplatin.

P-glycoprotein examination

P-glycoprotein is a protein that functions as a

biological barrier that secretes compounds that are toxic to cells. These proteins give cancer cells the ability to resist lethal doses of certain cytotoxic drugs by pumping the drugs out of the cells, thereby reducing their cytotoxicity. The higher the percentage of p-glycoprotein, the higher the cell's ability to resist the response of the given cytotoxic compound. In other words, if a low percentage of p-glycoprotein is found, then the cytotoxic ability of the drug has a positive effect on cells.

From Table 1, the mean value of p-glycoprotein levels in the control group is 31.67 ± 0.81, the mean value of p-glycoprotein levels in the doxorubicin group is 0 ± 0, the mean value of the IC50 cinnamon bark extract fraction is 12 ± 0.7, the mean value the IC50 cisplatin fraction group was 16 ± 1.59, the mean value of the combination IC50 cinnamon bark extract + IC50 cisplatin was 1.2 ± 0.1, the mean value of the combination IC50 cinnamon bark extract + IC50 cisplatin was 8.27 ± 0.15, the mean value the combination of IC50 cinnamon bark extract + IC50 cisplatin was 10.53 ± 0.81, and the mean value of the combination IC50 cinnamon bark extract + IC50 cisplatin was 13.57 ± 0.76. All treatments showed a significant value with $p < 0.001$. From the four treatment groups, the combination of IC50 cinnamon bark extract and IC50 cisplatin was able to lower p-glycoprotein levels higher with a lower mean value than other treatment groups.

From Figure 1, the average value of p-glycoprotein levels in the study group is obtained. The data in the image above show the highest p-glycoprotein expression in the control group of cells. The expression of p-glycoprotein in the control group of cells reached 31.67%. In the test group, the lowest p-glycoprotein expression was found in the combination 1 (1,2%). The expression of p-glycoprotein in the combination 1 and the doxorubicin group is shown in Figure 2.

Copper transporting ATPase 1 (CTR1) assay

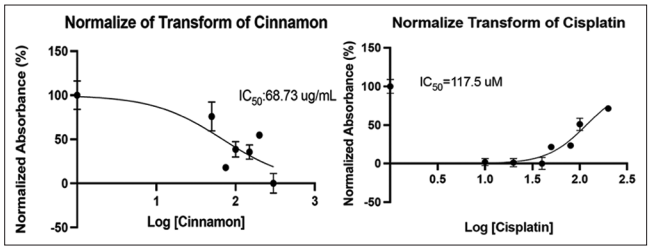

Figure 1: Normal levels of IC50

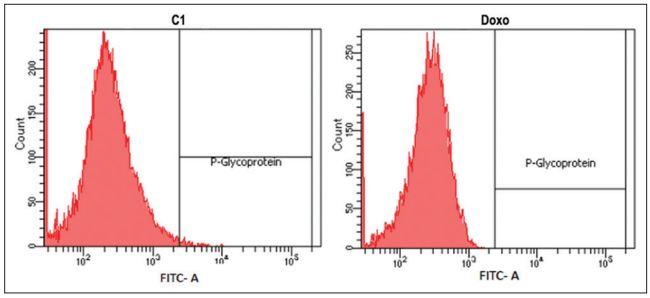

Figure 2: P-glycoprotein expression in combination 1 and doxo group measure using flow cytometry. Combination 1: Combination of 68,73 ug/ml cinnamon + 117,5 uM cisplatin; Doxo: Doxorubicin group.

CTR1 protein is known to correlate with tumor cell resistance to therapeutic agents, in this case, cisplatin. The lower the expression of CTR1, the higher the level of resistance of these tumor cells to therapeutic agents.

From Table 2, the mean value of CTR1 levels in the control group was 2.17 ± 0.21, the average value of CTR1 levels in the doxorubicin group was 29.13 ± 1.22, the average value of CTR1 levels in the IC50 cinnamon bark fraction group was 19.73 ± 0.49, the average value of CTR1 levels in the group the IC50 cisplatin fraction was 1.47 ± 0.6, the average value of CTR1 levels in the combination of IC50 cinnamon bark + IC50 cisplatin was 12 ± 0.92, the average value of CTR1 levels in the combination of IC50 cinnamon bark + IC50 cisplatin was 7.70 ± 1.15, the average value of the concentration of

AQ6 **Table 1: Levels of p-glycoprotein in the study group**

Group	P-Glycoprotein Mean ± SD	P-Value*	P-Value**						
			Control	Doxo	IC50 Cin	IC50 Cis	C1	C2	C3	C4
Control	31.67 ± 0.81	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Doxo	0 ± 0				< 0.001	< 0.001	1.00	< 0.001	< 0.001	< 0.001
IC50 Cin	12 ± 0.7					< 0.001	< 0.001	< 0.001	1.00	0.746
IC50 Cis	16 ± 1.59						< 0.001	< 0.001	< 0.001	0.045
C1	1.2 ± 0.1							< 0.001	< 0.001	< 0.001
C2	8.27 ± 0.15								0.078	< 0.001
C3	10.53 ± 0.81									0.006
C4	13.57 ± 0.76									

* One Way Annova
** Bonferroni
C1 : combination of 68,73 ug/ml Cinnamon + 117,5 uM Cisplatin
C2 : combination of 51,54 ug/ml Cinnamon + 88,13 uM Cisplatin
C3 : combination of 34,36 ug/ml Cinnamon + 58,75 uM Cisplatin
C4 : combination of 17,19 ug/ml Cinnamon + 29,38 uM Cisplatin
Cin : Cinnamon
Cis : Cisplatin
Doxo : Doxorubicin group

AQ6 **Table 2: CTR1 levels in the study group**

Group	CTR 1 Mean ±SD	P-Value*	P-Value**						
			Control	Doxo	IC50 Cis	IC50 Cis	C 1	C 2	C 3	C 4
Control	2.17 ±0.21	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Doxo	29.13 ±1.22				< 0.001	< 0.001	1.00	1.00	< 0.001	<0.001
IC50 Cin	19.73 ±0.49					< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
IC50 Cis	1.47 ±0.6						< 0.001	< 0.001	< 0.001	< 0.332
C 1	12 ±0.92							0.332	< 0.22	< 0.001
C 2	7.70 ±1.15								< 0.626	< 0.001
C 3	9.33 ±0.96									< 0.001
C 4	3.30 ±0.36									

* One Way Annova
** Bonferroni
C1 : combination of 68,73 ug/ml Cinnamon + 117,5 uM Cisplatin
C2 : combination of 51,54 ug/ml Cinnamon + 88,13 uM Cisplatin
C3 : combination of 34,36 ug/ml Cinnamon + 58,75 uM Cisplatin
C4 : combination of 17,19 ug/ml Cinnamon + 29,38 uM Cisplatin
Cin : Cinnamon
Cis : Cisplatin
Doxo : Doxorubicin group

CTR1 in the combination of IC50 cinnamon bark + IC50 cisplatin is 9.33 ± 0.96, and the average value of CTR1 levels in the combination of IC50 cinnamon bark + IC50 cisplatin is 3.30 ± 0.36. All treatments showed a significant value with $p < 0.001$. Of the four treatment groups, the combination of IC50 cinnamon bark and IC50 cisplatin had the highest CTR1 levels among the other three treatment groups.

The data in Figure 2 above show the lowest CTR1 expression in the control group of cells. The expression of CTR1 in the control group of cells reached 2.17%. In the test group, the highest CTR1 expression was found in the combination 1 test group, namely, the 1 × IC50 combination. The value of CTR1 expression in the combination group 1 was 12%. The expression of CTR1 in the combination test group 1 and the doxorubicin group is shown in Figure 3.

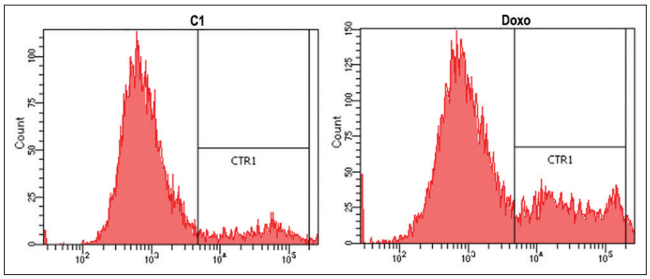

Figure 3: CTR-1 expression in combination 1 and doxo group measure using flow cytometry. Combination 1: Combination of 68,73 ug/ml cinnamon + 117,5 uM cisplatin; Doxo: Doxorubicin group.

Discussion

The use of platinum as an adjuvant therapy in ovarian cancer has long been used, but nowadays with the increasing rate of resistance and recurrence of ovarian cancer, it is necessary to find an alternative to reduce the resistance rate of platinum. Phytopharmaca is one of the alternatives that are starting to get a lot of attention, because it is easy to get, especially in Indonesia, which is a spice-producing country. Several previous studies have reported the anti-cancer effect of cinnamon on breast cancer with relatively good results. This opens new opportunities to try cinnamon on ovarian cancer, considering that the previous studies mentioned that the side effects of cinnamon are almost non-existent, although this still needs to be analyzed further and is still at the experimental stage using cell culture [8], [9], [10].

In this study, we found that the addition of cinnamon extract to cisplatin significantly reduced the expression of p-glycoprotein in SKOV3 cell cultures (Table 1), where we found the lowest p-glycoprotein expression at the IC50 dose of cisplatin and cinnamon extract. The suppressive effect on p-glycoprotein expression was found to decrease with decreasing doses of cisplatin and cinnamon extract. Based on this, we suspect that cinnamon extract can reduce the efflux of cisplatin from within the cells, thereby increasing the effectiveness of cinnamon to kill SKOV3 cancer cells, but this is influenced by the dose given, where the lower the dose given, the suppressive effect on drug efflux will also be more. P-glycoprotein was first identified in cancer cells. This protein is a protein that functions as a biological barrier that secretes compounds that are toxic to cancer cells. These proteins give cancer cells the ability to resist lethal doses of certain cytotoxic drugs by pumping the drugs out of the cells, thereby reducing their cytotoxicity. The higher the percentage of p-glycoprotein, the higher the cell's ability to resist the response of the given cytotoxic compound. In other words, if a low percentage of p-glycoprotein is found, then the cytotoxic ability of the drug has a positive effect on cells [11], [12], [13], [14], [15].

In cancer cells that are resistant to various anticancer drugs, the expression of p-glycoprotein is

increased, therefore p-glycoprotein is also known as a multidrug resistance protein. In addition to cancer cells, p-glycoprotein is also expressed in various non-tumor normal tissues with excretory function (small intestine, liver, and kidney) as well as on blood tissue barriers (blood–brain barrier, blood-testicular barrier, and placenta). The expression level of p-glycoprotein is illustrated from the results of the study (Figure 4). The control group had the highest p-glycoprotein expression reaching 31.67%. This indicates that in the control cell group, there is a release of therapeutic agents or drug efflux. This proves that SKOV3 cells are cisplatin-resistant cells. On the other hand, in the combination group, the p-glycoprotein expression tends to be 1.2% low. The low p-glycoprotein expression in the combination group indicated that there was no efflux of therapeutic agents or drugs in this case affecting resistance to cisplatin.

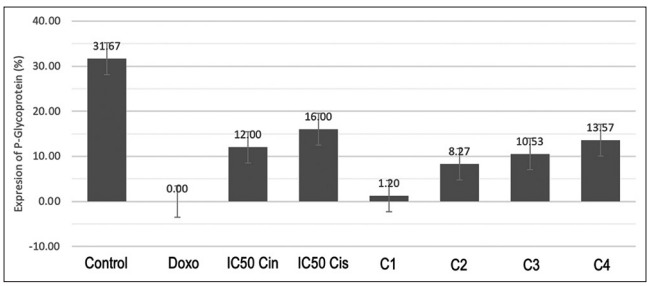

Figure 4: Comparison of p-glycoprotein levels in the study group. C1: Combination of 68.73 ug/ml cinnamon + 117.5 uM cisplatin; C2: Combination of 51.54 ug/ml cinnamon + 88.13 uM cisplatin; C3: Combination of 34.36 ug/ml cinnamon + 58.75 uM cisplatin; C4: Combination of 17.19 ug/ml cinnamon + 29.38 uM cisplatin; Cin: Cinnamon; Cis: Cisplatin; Doxo: Doxorubicin group.

Multidrug resistance (MDR) was synergistically inhibited by a combination of chemotherapy drugs and cinnamophilin. The lignan component of cinnamophilin from Cinnamomum Philippines significantly inhibited p-glycoprotein and resensitized MDR KB/VIN cancer. Cinnamophilin has two major effects on human p-glycoprotein. First, it can modulate efflux function through allosteric inhibition. Cinnamophilin non-competitively inhibits the transport of doxorubicin and rhodamine 123. Second, cinnamophilin on p-glycoprotein is associated with energy consumption. The p-glycoprotein substrate efflux is related to the energy consumption of ATP binding to NBD and hydrolysis. After ATP binding, ATP hydrolysis is essential for the conformational change of the p-glycoprotein from the outside to the inside that will continue the next efflux cycle [8], [9], [11], [15], [16]. This indicates that the combination of cinnamon bark extract of 68.73 ug/ml and cisplatin of 117.5 uM has the best effect on drug efflux so that it has the potential to prevent cisplatin resistance.

Uptake control of a therapeutic agent, one of which is regulated by the protein CTR1. Decreased cisplatin uptake by resistant cells is one of the main mechanisms of resistance described *in vitro*. The mechanism responsible for reducing cisplatin accumulation in resistant cells could be thought to be an inhibition of drug uptake, increased drug efflux, or both. Cisplatin and its analogs can accumulate in cells by passive diffusion or facilitated transport. Copper transporter-1 (CTR1) regulates the entry of cisplatin and its analogs into cells. This is supported by studies that remove the CTR1 gene in yeast, which encodes a high-affinity copper transporter, resulting in increased cisplatin resistance and reduced intracellular cisplatin accumulation in various cell types including ovarian cancer (Tapia and Diaz-Padilla, 2013). In human ovarian cancer cells, it is known that copper and cisplatin are competitive inhibitors of each other's transport into cells and cause downregulation of CTR1 expression through the internalization of the transporter of the plasma membrane [12], [14], [17], [18]. CTR1 protein is known to correlate with tumor cell resistance to therapeutic agents, in this case, cisplatin. The lower the expression of CTR1, the higher the level of resistance of these tumor cells to therapeutic agents. On the other hand, the higher the expression of CTR1, the more opportunities for cells to be invaded by therapeutic agents [19], [20], [21], [22], [23], [24], [25], [26], [27].

The expression level of CTR1 is illustrated from the results of the study (Figures 3 and 5). The control group had the lowest CTR1 expression which only reached 2.17%. This indicates that in the control cell group, cisplatin uptake was inhibited. On the other hand, in the combination group, CTR1 expression tends to be higher. The high expression of CTR1 in the combination group indicated good cisplatin uptake into cells. The high expression of CTR1 in the test group, namely, in the combination 1, indicated that the combination of cinnamon bark extract of 68.73 ug/ml and cisplatin of 117.5 uM had the best effect on the uptake of cisplatin therapeutic agents.

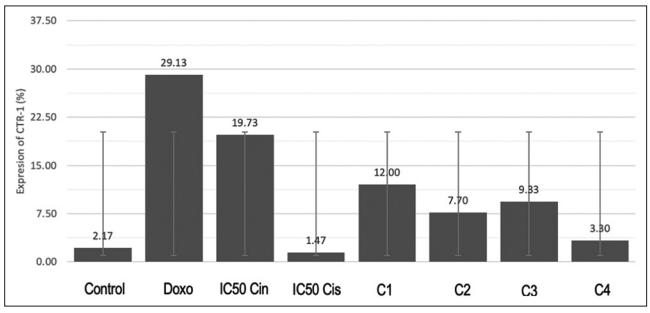

Figure 5: Comparison of CTR-1 levels in the study group. C1: Combination of 68.73 ug/ml cinnamon + 117.5 uM cisplatin; C2: Combination of 57.54 ug/ml cinnamon + 88.13 uM cisplatin; C3: Combination of 34.36 ug/ml cinnamon + 58.75 uM cisplatin; C4: Combination of 17.19 ug/ml cinnamon + 29.38 uM cisplatin; Cin: Cinnamon; Cis: Cisplatin; Doxo: Doxorubicin group; CTR-1: Copper transporting ATPase-1.

Conclusion

The combination of cinnamon bark extract with cisplatin was shown to decrease efflux by decreasing p-glycoprotein expression and increasing influx by increasing CTR1 expression in SKOV3 ovarian cancer cell cultures.

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