Effect of Trisindolina-5 Compound on Cancer Stem Cell (CSC) Proliferation in-Vitro

Sholeh Salispriaji[1], Awik Puji Dyah Nurhayati[1], Mardi Santoso[2], First Ambar Wati[2]

[1]Department of Biology, Institut Teknologi Sepuluh Nopember, Jl. Teknik Mesin No.173, Keputih, Sukolilo, Surabaya, East Java 60115.
[2]Department of Chemistry, Institut Teknologi Sepuluh Nopember, Jl. Teknik Mesin No.175, Keputih, Sukolilo, Surabaya, East Java 60115.

E-mail: nurawaliyah16@gmail.com

Abstract. Cancer stem cells (CSCs) are a subset of cancer cells that have the abilities of normal stem cells. CSCs are cancer cell pioneers with self-renewal abilities that can cause CSCs to differentiate into several cancer cells. Because CSCs are resistant to conventional therapies, killing CSCs necessitates the use of a compound with powerful anticancer properties. Trisindoline has been shown to have powerful anticancer properties. Trisindoline has been synthesized into several modifications, the most recent of which is Trisindoline-5. The goal of this study is to find out what the IC50 value of Trisindoline-5 is. The cytotoxicity assay using Microculture Tetrazolium Technique Assay (MTT Assay) is used to determine IC50. The IC50 value of the Trisindoline-5 compound is 24.683 µM at 24 hours incubation, which classifies it as a medium cytotoxic compound, 17.067 µM at 48 hours incubation, which classifies it as a highly toxic compound, and 6497 µM at 72 hours incubation, which classifies it as a compound with no toxicity. While the IC50 value of doxorubicin is 1.611 µM after 24 hours, 2.334 µM after 48 hours, and 5.324 µM after 72 hours, it is classified as a compound with highly toxic activity.

Keyword—CSC, Cytotoxicity, Doxorubicin, IC_{50}, MTT *assay*, Trisindoline

1. Introduction

Cancer stem cells (CSCs) or known as tumor-initiating cells (TICs) are a very small proportion of the cancer cell population that has the ability of normal stem cells [1]. CSCs are embryonic in nature which through both symmetric and asymmetric division can slowly maintain its population with the ability to self-renewal for a long period of time [2]. CSCs are also pioneers for cancer cells where this self-renewal ability will cause undifferentiated CSCs to differentiate into various types of cancer cells [3][4]. CSCs play a role not only in cancer formation but also metastasis, and recurrence [3]. CSCs metastasize by breaking away from the tumor mass and entering the bloodstream and circulating to parts of the body where they can invade other tissues and initiate the process of tumor formation. This metastatic ability is a major cause of cancer death [5][6].

A significant difference of cancer stem cells (CSCs) is their ability to be resistant to conventional

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

therapies [7]. Current therapies tend to center on killing rapidly dividing cells in the tumor mass. However, such therapies will spare the slower-dividing cancer stem cells (CSCs) that survive and can cause recurrence and regrowth of the tumor mass [8]. Therefore, CSCs are widely researched as the main target cells in cancer treatment [9].

Many cancer treatment strategies have been developed including surgery, radiotherapy, chemotherapy, and targeted therapy [10]. Surgery and radiotherapy are effective cancer treatments for non-metastatic cancers. As for metastatic cancer that attacks many organs of the body, the use of chemotherapy is more effective [8]. Besides being expensive, these treatments sometimes have side effects. So that treatment is developed that utilizes natural materials. The natural material used in previous studies is trisindoline. Trisindoline is a derivative compound of indole isolated from several natural sources including bacteria, sponges and plants. Indole compounds are widely used in the pharmaceutical world because they are highly toxic to microorganisms, mutagens and carcinogens [11].

Trisindoline-1 was first isolated from a culture of marine bacteria *Vibrio* sp. obtained from the Okinawan sea sponge *Hyrtios altum* [12]. Trisindoline itself has been synthesized into several modifications that have different toxic properties [13]. Previous research showed that trisindolina 1 has the highest cytotoxicity activity against MCF cancer cells with an IC_{50} value of 2.059 µM compared to other modifications [14]. After that, [15] revealed that the activity of Trisindolina 1 against CSCs had an IC_{50} value of 57.72 µM and correlated very strongly negatively with CSC viability. Then, [16] has synthesized the latest modification of trisindolina 5-floro-3,3-di((methyl indol-5- carboxylate)-3-yl)-2-indolone which has quite good cytotoxicity activity in DU-145 and A549 and is not toxic to h9c2 normal cells so it is very good to be used as an alternative anticancer drug compared to trisindoline-1 which is very toxic. Thus, further research was conducted to evaluate the cytotoxicity and determine the IC_{50} value of Trisindoline 5 against cancer stem cells (CSC) which are the initiation of various types of cancer.

2. Materials and Methods

2.1 Time and Place

This study was conducted during March 2022-June 2022. The research location was at the Stem Cell and Cancer Research (SCCR) Laboratory, Faculty of Medicine, Sultan Agung Islamic University Semarang, Semarang, Central Java.

2.2 Tools and Materials

The tools used were conical tube, 96-well plate, micropipette, tip, microtube, cryotube, flask, centrifuge, haemocytometer, CO2 incubator, Biological Safety Cabinet (BSC), ELISA reader, inverted microscope and liquid nitrogen tank. The materials used were Cancer Stem Cell (CSC) cultures, trisindoline compound powder, doxorubicin compound stock solution, Dulbecco's Modified Eagle's Medium (DMEM) F12, supplemental medium for mammosphere (MammoCult) 20%, fungizone (Amphotericin B) 0.25%, penicillin-streptomycin 1.24%, phosphate buffered saline (PBS), fetal bovine serum (FBS) 20%, glutamine 1.02%, trypsin-EDTA (ethylenediaminetetraacetic acid) 0.25%, dimethyl sulfoxide (DMSO) solvent, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) solution, sodium dodecyl sulfate (SDS-stopper) reagent, and 70% alcohol.

2.3 Methods

2.3.1 Preparation of Culture Medium

Preparation of complete culture medium was carried out in a biological safety cabinet (BSC) aseptically. Complete culture medium was prepared from a mixture of 20 ml 20% FBS, 20 ml 20% supplemented medium for mammosphere (MammoCult), 1.24 ml Penicillin-streptomycin 1.24% and 250 µl Amphotericin B 0.25% in a conical tube. DMEM F12 was added until the final volume in the conical tube was 100 ml. Then, the conical tube was labeled and stored at 2-8°C.

2.3.2 CSC Culture Preparation and Growth

Frozen cancer stem cell (CSC) cultures in cryotubes were taken from a liquid nitrogen tank at -80°C. Then thawing was done at room temperature. The thawed cell culture was then transferred into a sterile conical tube containing 3 ml of complete culture medium. Then, a centrifuge was performed on the cell suspension at 1000 rpm for 1-3 minutes, then the supernatant formed was discarded. Then in BSC cell culture, 4 ml of new complete medium was added and resuspended until homogeneous. Then added medium to a total of 7-10 ml and homogenized. Then grown to 80% confluence in a CO2 incubator at 37oC for 2-3 days. Every 24 hours of incubation the medium was replaced.

2.3.3 CSC Harvesting

The flask containing the cell culture is observed under an inverted microscope to see if it has reached 80% confluence. Cell culture that is 80% confluent indicates that harvesting can be done. The complete medium from the 80% confluent cell culture was removed from the flask using a micropipette. The remaining cell culture was washed with PBS twice. Then trypsin-EDTA 0.25% as much as 2-5 ml was added and incubated for 30 seconds in BSC followed by incubation for 3 minutes in a CO2 incubator at 37˚C to release cells from the flask wall and become single cells. Then 5-7 ml of trypsin inactivation medium was added to the flask and the cells were resuspended until the cells were detached from the flask wall. To ensure that the cells were completely detached and not clumped, the cells were observed again with an inverted microscope. If the cells were still clumped then resuspension was done again. If the cells were detached and not clumped then the cells were transferred into a sterile conical tube and labeled.

2.3.4 CSC Counting

The harvested cells in the conical tube were taken as 10 µl cell harvest to count the number of cells using a haemocytometer. Cells were transferred into the haemocytometer using a micropipette and counted with a counter under an inverted microscope.

Calculations were carried out in four counting rooms (Room W) with the stipulated conditions, namely dark cells (dead) and cells on the left and upper outer boundaries were not counted, while cells on the right and lower outer boundaries were counted. Then totaled using the following formula:

$$\Sigma \text{ cell/ml} =((\Sigma \text{ cell in chamber W})/4) \times 10^4 \quad (1)$$

The total number of cells required was calculated, for a 96-well plate, the total number of cells required was 5x103 cells/well x 100 wells, resulting in 5x105 cells. Then the required volume of harvested cells (in ml) is calculated with the following calculation formula:

$$\text{Harvested cells volume} = (\text{total number of cells required})/(\text{number of cells counted/ml}) \quad (2)$$

The cell harvest volume is transferred to a new conical tube and culture medium is added until the total volume required. The calculation of the required volume is that each well will be filled with 100 µL of culture medium containing cells, so the total volume needed to plant cells in 96-well plates is 100 µL x 100 wells resulting in 10 ml.

2.3.5 Compound Preparation

As much as 5 mg of trisindoline powder was mixed with DMSO in a microtube to obtain a trisindoline stock solution with a concentration of 100,000 µM. Furthermore, dilutions were made with concentration series of 1 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL.

Doxorubicin stock solution was made with a concentration of 100,000 µM. Furthermore, dilutions were made with concentration series of 0.5 µg/mL, 1 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL, 25 µg/mL, and 50 µg/mL.

For each concentration series made will be placed in a separate microtube and replicated twice. Each microtube series of trisindolina compound solution is used for 3 wells so that if there are two

replications, the number of wells filled is 6 wells for each concentration.

2.3.6 Cytotoxicity Assay

The cytotoxicity assay was performed using the MTT assays method. The cell culture was distributed to 96-well plates with $5x10^3$ cells/100 µL in each well, then incubated for 24 hours at 37°C in a CO_2 incubator until confluent. After confluent (ready to be treated), the plate was taken from the CO_2 incubator to be taken to the BSC. The culture medium was discarded then with 100 µL PBS all wells filled with cells were washed then PBS was discarded and the remaining liquid was drained with a tissue. The concentration series of samples were put into the wells and incubated in a CO_2 incubator with different incubation times of 24 hours, 48 hours, and 72 hours.

After incubation, cell conditions were observed and documented for each treatment group, except media control. MTT powder as much as 50 mg was weighed and dissolved in 10 ml PBS, then stored in a freezer covered with aluminum foil. Then 1 ml of MTT stock was taken and diluted with culture medium to 10 ml (for each 96-well plate). The culture medium was discarded and then washed with PBS and 100 µL of MTT reagent was added to each well, including control media. Then incubated again in a CO_2 incubator for 2-4 hours. Cell conditions were examined with an inverted microscope. If formazan formed, 100 µl of stopper in the form of 10% SDS solution in 0.01 N HCl was added to each well to dissolve the formazan crystals. The plate was then wrapped with aluminum foil and incubated in a dark place at room temperature overnight.

The ELISA reader was turned on and waited until the progressing process was complete. The wrapper and plate lid were opened and inserted into the ELISA reader at a light wave of 595 nm. Then the start button was pressed and the absorbance results of each well appeared. After completion, the ELISA reader is turned off and the results are stored. Furthermore, the percentage of cell viability was calculated and then the IC_{50} value was analyzed by probit regression analysis of SPSS Statistic 25 software.

3. Results and Discussion

3.1 Morphological Observation of CSC

Observations of Cancer Stem Cell (CSC) morphology after treatment with Trisindolina 5, Doxorubicin, and untreated control cells were observed using an inverted microscope presented in Figure 4.1.

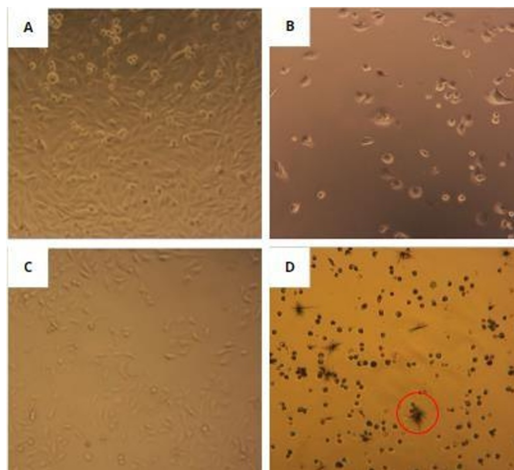

Figure 1. Morphology of CSCs treated with (A) cell control, (B) Trisindolina 5, and (C) Doxorubicin, (D) formation of formazan crystals (Phase contrast, x40)

Observations showed morphological changes in treated cells compared to untreated control cells. In

normal cells, Figure 1.A shows a spindle morphology and strong intercellular adhesion. Adhesion between cells causes CSCs to adhere to each other and form a tight collection of cells. Normal CSCs are classified as adherent or grow attached to the surface of the culture container. Cells treated with Trisindolina 5 in Figure 1.B and Doxorubicin treatment in Figure 1.C show changes in cell morphology where cells look round. This indicates the occurrence of apoptosis where chromatin condensation and nucleus fragmentation occurs so that cell morphology appears round and experiences shrinkage/reduction of cellular volume or pycnosis [17]. In addition, intercellular adhesion is also drastically reduced when compared to control cells. When the compound can damage cell adhesion, the cells will be spherical/circular. This is due to damage to the extracellular matrix (ECM) where the ECM is what provides elastic properties, span, and cell compaction [18]. Meanwhile, Figure 1.D shows the formation of a purplish blue formazan crystal structure that indicates the reduction of MTT by succinate dehydrogenase contained in the mitochondria of living cells [19].

3.2 Trisindoline 5 Cytotoxicity Assay

The cytotoxicity assay of Trisindoline 5 was performed using the MTT method with varying incubation times of 24, 48, and 72 hours. The incubation time variation was used because the cell cycle length varies from 22 to 36 hours and is usually 24 hours in human cells. The absorbance values of cells treated with the test compound Trisindolina 5 are shown in Table 1, Table 2, and Table 3. These values were then used to determine the percentage of live cells (viability). Furthermore, probit analysis was conducted using SPSS Statistic 25 application to determine the IC_{50} value.

Table 1. Trisindoline 5 cytotoxicity assay for 24 hours of incubation

Concentration	**Absorbance** Average	**% Viability**	**% Inhibition**
2,5	0,141	124	0
5	0,131	66	34
25	0,130	65	35
50	0,127	44	56
100	0,119	5	95
Control Cell	0,137	-	
Control Medium	0,118	-	
IC_{50} SPSS	24,683 µM		

Table 2. Trisindoline 5 cytotoxicity assay for 48 hours of incubation

Concentration	**Absorbance** Average	**% Viability**	**% Inhibition**
2,5	0,113	100	0
5	0,107	68	32
25	0,101	34	66
50	0,100	28	72
100	0,097	13	87
Control Cell	0,113	-	
Control Medium	0,095	-	
IC_{50} SPSS	17,067 µM		

Table 3. Trisindoline 5 cytotoxicity assay for 72 hours of incubation

Concentration	**Absorbance**	**%**	**%**

	Average	Viability	Inhibition
2,5	0,093	97	3
5	0,099	183	0
25	0,092	87	13
50	0,102	225	0
100	0,092	83	17
Control Cell	0,093	-	
Control Medium	0,086	-	
IC_{50} SPSS	6497 µM		

The IC_{50} value of Trisindoline 5 at the 24-hour incubation time was 24.683 µM which according to the National Cancer Institute (NCI) criteria included moderate cytotoxic criteria. The IC_{50} value of Trisindoline 5 at an incubation time of 48 hours amounted to 17.067 µM which according to the National Cancer Institute (NCI) criteria included highly toxic criteria. Meanwhile, the IC_{50} value of Trisindoline 5 at 72 hours incubation time was 6497 µM which according to the National Cancer Institute (NCI) criteria included non-toxic criteria. The IC_{50} value at each time on Trisindoline 5 showed a decrease in IC_{50} at 48 hours and an increase in IC_{50} at 72 hours. The increase in IC_{50} at 72 hours indicates a decrease in the cytotoxicity activity of the Trisindoline 5 compound and indicates that the effectiveness of Trisindoline 5 decreases when the CSC incubation incubation is prolonged.

Based on the data of cancer stem cell (CSC) live cell percentage with the treatment of the test compound Trisindolina 5, the probit curves are shown in Figure 2, Figure 3, and Figure 4.

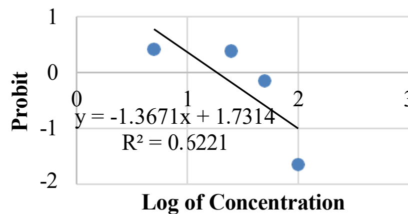

Figure 2. Probit results of Trisindoline 5 cytotoxicity against CSC after 24 hours of incubation

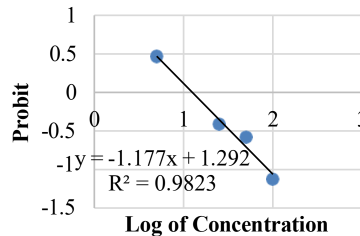

Figure 3. Probit results of Trisindoline 5 cytotoxicity against CSC after 48 hours of incubation

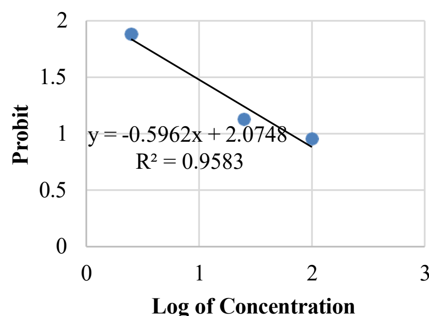

Figure 4. Probit results of Trisindoline 5 cytotoxicity against CSC after 72 hours of incubation

Furthermore, regression analysis through graphs and the coefficient of determination (R^2) showed different values for each variation of incubation time. The negative sign in each equation indicates that the movement of the values of variables X and Y is not in the same direction, meaning that the higher the concentration of Trisindoline 5 given, the lower the percentage of live cells. The coefficient of determination can be seen through the R-square (R^2) value which shows the extent to which the contribution of the independent variables in the regression model is able to explain the variation of the dependent variable. When the independent variables are combined with the dependent variable, the coefficient of determination (R^2) is used to determine and predict the magnitude or significance of the influence provided by the independent variables. The coefficient of determination can vary from 0 to 1. If the value is close to one, the independent variables provide almost all of the information required to estimate the dependent variable. However, as the R^2 value decreases, it indicates that the ability of the independent variables to explain the dependent variable becomes more limited [20].

The decrease in cytotoxicity activity and effectiveness of Trisindoline 5 is because CSCs have a relatively weak sensitivity to Trisindoline 5. The weak sensitivity of CSCs to anticancer compounds is due to the characteristics of CSCs which are known to have therapeutic resistance capabilities [1][21][22]. This is because CSCs express transmembrane transporters, such as the ABC (ATP-binding cassette) transporter family, which are able to actively pump drug molecules out of the cell. CSCs also have enzymes, such as aldehyde dehydrogenase (ALDH), and glutathione transferase (GST), which are able to metabolize and inactivate anticancer agents. In addition, CSCs also have increased expression of anti-apoptotic proteins, such as Bcl-2 and Bcl-X. CSCs are also able to promote DNA damage repair by activation of DNA damage checkpoints such as CHK1 and CHK2, as well as activation of pro-survival signaling molecules [1][23]. In addition, several signaling pathways in CSCs are also known to be associated with therapeutic resistance, for example the WnT, Notch, and Hedgedog signaling pathways that regulate self-renewal and differentiation in CSCs [24][25].

Trisindoline 5 is a compound that has an indole molecule with the modification of adding a side group in the form of a fluorine group that serves to increase its potential as an anticancer compound [26]. The fluorine group is on carbon number 5 of the isatin molecule [16]. The isatin molecule is an oxidized derivative of the indole molecule which is able to induce cell death in the form of both apoptosis and necrosis [27]. Isatin is known to inhibit cancer cell proliferation through interaction with extracellulear signal-related protein kinases (ERK) so as to trigger apoptosis. ERK is able to activate apoptosis, both extrinsic and intrinsic pathways [28]. The mechanism of cytotoxicity of fluorine groups in Trisindolina 5 compounds is still unknown. However, many additions of fluorine groups to medicinal compounds have proven cytotoxicity mechanisms, for example, the antitumor drug 5-fluorouracil (5-FU) which works by converting to active metabolites that inhibit the enzyme thymidylate synthase, an enzyme that converts 20-deoxyuridine-50-monophosphate (dUMP) into 20-deoxythymidine-50 monophosphate (dTMP) and causes a decrease in thymidine formation so that it

will reduce DNA synthesis [29][30]. Therefore, the presence of isatin molecules and fluorine groups in the Trisindoline 5 compound allows this compound to be toxic to cancer cells.

3.3 Doxorubicin Cytotoxicity Assay

Doxorubicin cytotoxicity assay was conducted using MTT method with variation of incubation time of 24, 48, and 72 hours as positive control. The absorbance value of cells treated with Doxorubicin test compound is shown in Table 4, Table 5, and Table 6. The value was then used to determine the percentage of living cells (viability). Furthermore, probit analysis was conducted using SPSS Statistic 25 software to determine the IC_{50} value.

Table 4. Doxorubicin cytotoxicity assay results for 24 hours incubation

Concentration	Absorbance Average	**% Viability**	**% Inhibition**
0,5	0,361	83	17
1	0,322	38	62
5	0,312	25	75
25	0,307	20	80
50	0,319	34	66
Control Cell	0,375	-	
Control Medium	0,290	-	
IC_{50} SPSS	1,611 μM		

Table 5. Doxorubicin cytotoxicity assay results for 48 hours incubation

Concentration	Absorbance Average	**% Viability**	**% Inhibition**
0,5	0,395	128	0
1	0,286	15	85
5	0,316	46	54
25	0,312	42	58
50	0,278	7	93
Control Cell	0,271	-	
Control Medium	0,368	-	
IC_{50} SPSS	2,335 μM		

Table 6. Doxorubicin cytotoxicity assay results for 72 hours incubation

Concentration	Absorbance Average	**% Viability**	**% Inhibition**
0,5	0,318	159	0
1	0,296	140	0
5	0,167	26	73
25	0,155	15	85
50	0,146	7	93
Control cell	0,251	-	
Control Medium	0,138	-	
IC_{50} SPSS	5,324 μM		

The IC_{50} value of Doxorubicin at the 24-hour incubation time was 1.611 μM, at the 48-hour incubation time was 2.335 μM and at the 72-hour incubation time was 5.324 μM which according to the National Cancer Institute (NCI) criteria includes highly toxic criteria. The IC_{50} value of the Doxorubicin compound is smaller than the IC_{50} value of Trisindoline 5. Indicates that the activity and

effectiveness of Doxorubicin is better than Trisindoline 5. So it is proven that Doxorubicin as a commercial drug has stable effectiveness and its cytotoxic activity is still very toxic even though the incubation is prolonged.

Based on the data of cancer stem cell (CSC) live cell percentage with the treatment of Doxorubicin test compound, the probit curves are shown in Figure 5, Figure 6, and Figure 7.

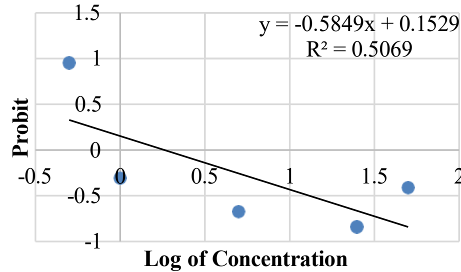

Figure 5. Doxorubicin cytotoxicity probit results against CSC after 24 hours of incubation

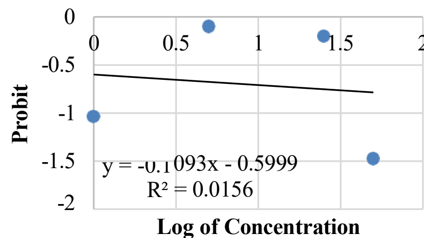

Figure 6. Doxorubicin cytotoxicity probit results against CSC after 48 hours of incubation

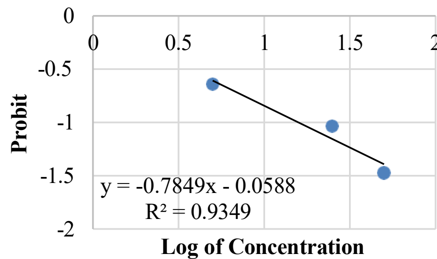

Figure 7. Doxorubicin cytotoxicity probit results against CSC after 72 hours of incubation

Furthermore, regression analysis through graphs and the coefficient of determination (R^2) showed different values for each variation of incubation time. The negative sign in each equation indicates that the movement of the values of the X and Y variables is not in the same direction, meaning that the higher the concentration of Doxorubicin given, the lower the percentage of living cells.

The anticancer activity of Doxorubicin is known to intercalate with DNA and inhibit topoisomerase II (TOP2) of cancer cells resulting in inhibition of DNA and RNA synthesis, thus activating p53 to perform cell cycle arrest and if it cannot be repaired it will stimulate apoptosis [31][32]. In addition, Doxorubicin also forms semiquinone free radicals and reactive oxygen species (ROS). ROS can cause adverse effects in the form of lipid peroxidation and membrane damage, DNA damage, oxidative stress and cause cells to undergo apoptosis [31].

4. Conclusion

From the research that has been carried out, it can be concluded that the IC_{50} value of Trisindolina 5 at the 24-hour incubation time of 24.683 μM includes moderate cytotoxic criteria, at the 48-hour incubation time of 17.067 μM including highly toxic criteria, at the 72-hour incubation time of 6497 μM including non-toxic criteria. The IC_{50} value of doxorubicin is 1.611 μM at 24 hours incubation, 2.335 μM at 48 hours incubation, and 5.324 μM at 72 hours incubation so it is classified as a compound with highly toxic activity.

Acknowledge

The authors would like to express their appreciation to the Department of Biology FSAD ITS for financing research in 2022, as well as the Directorate of Higher Education, Ministry of Education and Culture of the Republic of Indonesia, for funding through the Bidik Misi Scholarship 2018-2022.

References

[1] A. Z. Ayoub, and T. S. Ramasamy, "Cancer stem cells askey drivers of tumour progression," *J. Biomed. Sci*, Vol 25 (2018) 1-18
[2] L. Shahriyani, and N. L. Komarova, "Symmetric vs. asymmetric stem cell divisions: An adaptation against cancer?," *PLoS ONE*. Vol. 8 No 10 (2013)
[3] R. B. Moharil, A. Dive, S. Khandekar, and A. Bodhade, "Cancer stem cells: An insight," *J. Oral Maxillofac Pathol*. Vol. 21 (2017)
[4] X. Zheng,C. Yu, and M. Xu, "Linking tumor microenvironment to plasticity of cancer stem cells: Mechanisms and application in cancer therapy," *Frontiers in Oncology*. Vol. 11 (2021)
[5] M. Prieto-Vila, R. Takahashi, W. Usuba, I. Kahoma, and T. Ochiya, "Drug resistance driven by cancer stem cells and their niche," *International Journal of Molecular Sciences.* Vol. 18 (2017) 1-22
[6] M. Sreepadmanabh, and B. J. Toley, "Investigations into the cancer stem cell niche using in-vitro 3-D tumor models and microfluidics," *Biotechnology Advances.* Vol. 36, No. 4 (2018) 1094-1110
[7] L. Walcher, A. K. Kistenmacher, H. Suo, R. Kitte, S. Dluczek, A. Strauß, A. R. Blaudszun, T. Yevsa, S. Fricke, and U. Kossatz-Boehlert, "Cancer stem cells – Origin and biomarkers: Perspectives for targeted personalized therapies," *Front. Immunol*. Vol. 11 (2020) 1-13
[8] L. Yang, P. Shi, G. Zhao, J. Xu, W. Peng, J. Zhang, G. Zhang, X. Wang, Z. Dong, F. Chen, and H. Cui, "Targeting cancer stem cell pathways for cancer therapy," *Signal Transduction and Targeted Therapy*. Vol. 5 (2020)
[9] Y. Zhao, Q. Dong, J. Li, K. Zhang, J. Qin, J. Zhao, Q. Sun, Z. Wang, T. Wartmann, K. W. Jauch, P. J. Nelson, L. Qin, and C. Burns, "Targeting cancer stem cells and their niche: perspectives for future therapeutic targets and strategies," *Seminars in Cancer Biology*. Vol. 53 (2018) 139-155
[10] R. Baskar, J. Dai, N. Wenlong, R. Yeo, and K. Yeoh, "Biological response of cancer cells to radiation treatment," *Frontiers in Molecular Biosciences*. Vol. 1 No. 24 (2014)
[11] F. A. Wati, "Pengembangan Senyawa-Senyawa Heterosiklik Nitrogen sebagai Antituberkolosis dan Antikanker". *Disertasi*. Program Pendidikan S3 Kimia, Institut Teknologi Sepuluh Nopember: Surabaya (2021)
[12] M. Kobayashi, S. Aoki, K. Gato, K. Matsunasmi, M. Kurosu, and I. Kitagawa, "Marine natural. products XXXIV Trisindoline, a new abiotic indole primer by a bacterium of *Vibrio* sp. separated from the marine sponge *Hyrtios altum*," *Chem Pharm Bull*. Vol. 42 No. 12 (1994) 2449-2451
[13] Y. Mursyidah, and M. Santoso, "Sintesis dan sitotoksisitas 5-bromo-3-hidroksi-3-(1H-indol-3-il)indoline-2-one dan 3-hidroksi-5-kloro-1-metil-3-(1H-indol-3-il)indoline-2-one terhadap sel kanker serviks HeLa dan sel kanker kolon WiDr," *Prosiding Kimia*. Institut Teknologi Sepuluh Nopember (2010)
[14] L. Pertiwi, "Uji sitotoksisitas empat Trisindolina terhadap kanker payudara MCF-7 cell line," *Undergraduate Thesis*. Institut Teknologi Sepuluh Nopember (2017)

[15] F. A. Rahman, “Aktivitas senyawa Trisindolina 1 terhadap kadar reactive oxygen species (ROS) pada cancer stem cell,” *Undergrad. Thesis*. Institut Teknologi Sepuluh Nopember (2021)
[16] F. A. Wati, M. Santoso, Z. Moussa, S. Fatmawati, A. Fadlan, and Z. M. A. Judeh, “Chemistry of trisindolines: natural occurrence, synthesis and bioactivity,” *RSC Advances*. Vol. 11 (2021)
[17] G. Kroemer, W. S. El-Deiry, M. E. Peter, D. Vaux, P. Vandenebeele, B. Zhivotovsky, M. V. Blagosklonny, W. Malomi, R. A. Knight, M. Piacentini, S. Nagata, and G. Melino, “Classification of cell death; Recommendations of the nomenclature commitee on cell death,” *Cell Death and Differentiation*. Vol. 12 (2005) 1463-1467
[18] K. Y. Deleon, A. Yabluchanskiy, M. D. Winniford, R. A. Lange, R. J. Chilton, and M. L. Lindsey, “Modifying matrix remodeling to prevent heart failure,” *Woodhead Publishing Limited.* (2014)
[19] R. Dona, N. Sulistyani, and H. L. Nurani, “Uji sitotoksisitas dan antiproliferatif ekstrak etanol daun (*Solanum nigrum*, L.) terhadap sel raji,” *Pharmaciana*. Vol. 6 No. 2 (2016)
[20] I. Ghozali, *Aplikasi analisis multivariete dengan program IBM SPSS 23 edisi 8*. Jogjakarta: Badan Penerbit Universitas Diponegoro (2016)
[21] H. Dana, G. Mahmoodi, V. Marmari, A. Mazraeh, and M. Ebrahimi, “An overview of cancer stem cell,” *Journal of Stem Cell Research & Therapeutics*. Vol. 1 No. 4 (2016) 169-174
[22] F. Rossi, H. Noren, R. Jove, V. Beljanski, and K. H. Grinnemo, “Differences and similarities between cancer and somatic stem cells: Therapeutic implications,” *Stem Cell Research & Therapy*. Vol. 11 No. 489 (2020) 1-16
[23] C. Zhang, Z. Yang, D. L. Dong, T. S. Jang, J. C. Knowles, H. W. Kim, G. Z. Jin, and Y. Xuan, “3D culture technologies of cancer stem cells: Promising ex vivo tumor models,” *Journal of Tissue Engineering*. Vol. 11 (2018) 1-17
[24] B. W. L. Lee, P. Ghode, and D. S. T. Ong, “Redox regulation of cell state and fate,” *Redox Biol.* Vol. 25 (2019) 1-8
[25] F. Papaccio, F. Paino, T. Regad, G. Papaccio, V. Desiderio, and V. Tirino, “Concise review: cancer cells, cancer stem cells, and mesenchymal stem cells: influence in cancer development,” *Stem Cells Translational Medicine*. Vol. 6 (2017) 2115–2125
[26] E. H Hariyanti, and M. Santoso, “Sintesis dan uji sitotoksisitas 5-fluoro-3-hidroksi-3-(1h-indol-3-il)indolin-2-on dan 3-hidroksi-5-fluoro-1-metil-3-(1h-indol-3-il)indolin-2-on terhadap sel WiDr dan HeLa,” *Prosiding Kimia*. Institut Teknologi Sepuluh Nopember (2010)
[27] A. Medvedev, O. Buneeva, and V. Glover, Biological targets for isatin and its analogues: Implications for therapy,” *Biologics: Targets & Therapy*. Vol. 1 No. 2 (2007) 151-162
[28] K. L. VIne, L. Matesic, J. M. Locke, and D. Scropeta, “Recent highlights in the development of isatin-based anticancer agents,” *Advances in Anticancer Agents in Medicinal Chemistry*. Vol. 2 (2013) 254-312.
[29] C. Isanbor, and D. O’Hagan, “Fluorine in medicinal chemistry: A review of anti-cancer agents,” *Journal of Fluorine Chemistry*. Vol. 127 (2006) 303-319
[30] P. Shah, and A. D. Westwell, “The role of fluorine in medicinal chemistry,” *Journal of Enzyme Inhibition and Medicinal Chemistry*. Vol. 22 No. 5 (2007) 527-540
[31] C. F. Thorn, C. Oshiro, S. Marsh, T. Hernandez-Boussard, H. McLeod, T. E. Klein, & R. B. Altman, “Doxorubicin pathways: Pharmacodynamics and adverse effect,” *Journal of Pharmacogenet Genomics*. Vol. 21 No. 7 (2011)
[32] F. Yang, S. S. Teves, C. J. Kemp, & S. Henikoff, “Doxorubicin, DNA torsion and chromatin dynamics,” *Biochimica et Biophysica Acta Journal*. Vol. 1 (2014) 84-89