JPPIPA 10(8) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Prostate Anticancer Activity Testing α Mangostin Invitro Study on Sel DU 145 Using WST 8 Method

Rai Chika Dewi[1], Syafika Alaydrus[1*], Tien Wahyuhandayani[1]

[1] Departments of Pharmacology and Clinical Pharmacy, STIFA Pelita Mas Palu, Palu, Indonesia

Received: July 4, 2024
Revised: August 15, 2024
Accepted: August 25, 2024
Published: August 31, 2024

Corresponding Author:
Syafika Alaydrus
syafikaalaydrus39@gmail.com

DOI: 10.29303/jppipa.v10i8.8517

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Abstract: Prostate cancer is a cancer that has a high risk of death. Prostate cancer, specifically prostate adenocarcinoma, arises from the cells in the prostate gland or its peripheral zone. Chemotherapy, radiation therapy, and surgery are the main treatments for prostate cancer today. Empirical evidence shows that α-mangostin is used as a cancer therapy. At every stage of carcinogenesis, including cell division, proliferation, apoptosis, inflammation, and metastasis, α-mangostin can inhibit it. This study aims to evaluate the activity of α-mangostin against the DU145 prostate cancer cell line, and compare the %survival rate between cisplatin and α-mangostin. The method used is cytotoxicity testing in vitro with the WST-8 method. The research results showed that α-mangostin had an IC50 value of 16.89 ppm, in the active category, IC50 of cisplatin was 4.69 in the active category. Then statistical analysis was carried out using GraphPad Prism versi9.0.0 The results obtained were no significant differences between %survival rate cisplatin and α-mangostin at a concentration of 12.5 ppm. The conclusion of this study is The IC50 values of α-Mangostin and cisplatin against DU 145 cells were 16.89 ppm and 4.65 ppm, respectively. At a concentration of 12.5 ppm, there was no significant difference in their effects.

Keywords: α- mangostin; Cisplatin; Prostate cancer

Introduction

Prostate cancer is a cancer that has a high risk of death (Murtaza et al., 2020). Prostate cancer is cancer that originates from the cells of the prostate gland or peripheral zone, known as prostate adenocarcinoma (Zhong et al., 2023). According to Cabasag et al. (2022), in men, prostate cancer is a type of cancer that often occurs, with an incidence in Indonesia of 13,563 with a death toll of 4,863 (Cabasag et al., 2022).

Chemotherapy, radiation therapy, and surgery are the main treatments for prostate cancer today (Andriese et al., 2023). Conventional chemotherapy still has many disadvantages, including adverse effects on healthy tissue and relatively rapid drug metabolism before the agent reaches the tumor site (Muchtaridi & Wijaya, 2017). Additionally, because metastasis is common in prostate cancer cases, surgery often provides negligible results (Kamijima et al., 2022). Prostate cancer treatment technology still needs to be innovative to achieve better therapeutic results with fewer side effects.

A number of studies have examined the effectiveness of natural compounds as anticancer agents, and α-mangostin is one such ingredient. The yellow chemical known as α-mangostin is soluble in methanol, ethanol, ether, acetone, ethyl acetate, and chloroform but not in water. The chemical formula of α-mangostin is C24H26O6, and its molecular weight is 410.4596 g/mol. (Wathoni et al., 2020).

Extensive research has been conducted on the anticancer properties of α-mangostin on cancer cell types in vitro and in vivo. The results were extraordinary, α-mangostin showed maximal inhibition at a concentration of 10 µM as well as the ability to trigger programmed cell death or cell apoptosis in leukemia cell lines. (HL60), meanwhile, experiments conducted on mice in vivo showed that α-mangostin had a chemopreventive impact on colon carcinogenesis (Shibata et al., 2011). In addition, α-mangostin is able to induce apoptosis in cells associated with pancreatic cancer (PCL12), colon cancer (DLD-1), and breast cancer (BC-1, MCF-7, MDA-MB-231), Skin cancer (KB), lung

How to Cite:
Dewi, R. C., Alaydrus, S., & Wahyuhandayani, T. (2024). Prostate Anticancer Activity Testing α Mangostin Invitro Study on Sel DU 145 Using WST 8 Method. *Jurnal Penelitian Pendidikan IPA*, *10*(8), 5851–5855. https://doi.org/10.29303/jppipa.v10i8.8517

cancer (A549), head and neck squamous cell carcinoma (HNSCC), human melanoma cell line (SK-MEL-28), breast cancer, bone cancer (canine osteosarcoma D-17), colorectal cancer (HCT-116), and the ability to prevent the spread of prostate cancer (Pc-3), and other types of cancer cells through different (many) pathways, such as the mitochondrial pathway, JNK signaling, regulation of the β catenin gene in the Wnt/cGMP pathway, AKT /PI3K, MAPK, and αvβ3 Integrin/FAK/ERK signaling (Patil et al., 2021; Rusdin, 2019; Vien et al., 2021). Keeping this context in mind, the research was conducted.

Method

Location and Time of Research

This research will start from July 2024 until completion. Research location at the Cell and Molecular Biology Laboratory, Faculty of Pharmacy, Padjadjaran University.

Research Methode

Utilizing in vitro experiments on the DU145 prostate cancer cell line, this study tested the potential anti-prostate cancer effects of α-mangostin in the laboratory.

Tools and Materials

Tools

Tweezers, chamber, measuring cup (Pyrex), ruler, analytical balance (Precisa), dropper pipette, beaker (Pyrex), test tube (Pyrex), Erlenmeyer (Pyrex), microplate reader with a 450-490 nm, 96 well microplate, 6 well plate (Nest), multichannel pipette (8 or 12 channels ; 10-100 µl), 100 mm plate (Nest), serological pipette 10 ml, 25 ml, 5 ml (Nest), micropipette (Eppendorf) various sizes and Pipette tips size 200 µl (GenFollower), 1000 µl (GenFollower).

Material

The test material, namely α-mangostin, is a collection from the Pharmacy and Technology Laboratory at Padjadjaran University and the DU145 cell line is a collection from the Cell and Molecular Biology Laboratory at Padjadjaran University, WST-8, PBS, Penicillin-Streptomycin (Sigma), Fetal Bovine Serum (Sigma), Phosphate Buffer Saline 10X (Lonza) Trypsin TrypLE (Gibco), Dulbecco's Modified Eagle Medium (DMEM), high glucose (Sigma).

Preparation of Test Materials

Preparation of Test Materials a-Mangsotin

The α- Mangostin used in this research came from Punca Loka Nusantara, the α- mangostin obtained had a purity of <95%. In this test the doses used were 25 ppm, 50 ppm and 12.5 ppm.

In Vitro Activity Testing

Making Medium (100 ml Medium)

Take 90% Dulbecco's Modified Eagle Medium (DMEM), 1% penicillin-streptomycin (PS) in the chiller and 10% Fetal Bovine serum (FBS) in the freezer. Then spray it with 70% alcohol, then put it in a water bath at a temperature of 370C maximum 2 minutes. After that, spray it again with 70% alcohol and put it in Bio Safety Cabinet (BSC). Then carry out the treatment, namely entering penicillin-streptomycin (PS) as much as 1 ml, Fetal Bovine serum (FBS) as much as 10 ml and Dulbecco's Modified Eagle Medium (DMEM) 90 ml into the bottle. then homogenize with a pipette by moving up and down. Then spray again with 70% alcohol. After that enter penicillin-streptomycin (PS), Dulbecco's Modified Eagle Medium (DMEM), into the chiller and Fetal Bovine serum (FBS) in the freezer.

Culture Sel

Prepare DMSO and put 1 ml of media in the freezer until it freezes, then remove the cells to -80°C (Wait until they are liquid), then transfer 1 ml of cells to a 15 ml cup, then add 3 ml of media, centrifuge for 4 minutes then discard the media , suspended with 1 ml of media (homogenize by pipetting up and down), then put the media into the petri dish until it covers the surface of the dish, then put the cells into the media evenly and cover the petri dish then slide it right and left to make it homogeneous, then incubate media into the incubator for 24 hours.

Cytotoxic Activity Testing Using the WST 8

The test material was tested against the DU145 prostate cancer cell line using the method WST-8. Cell lines were cultured using Dulbecco's Modified Eagle Medium which contains 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were seeded on 96-well plates plate and incubated for 1 day with CO levels2 5% at 37°C. After that, the culture medium was replaced with new one and samples were given with several variations in concentration, Cisplatin as a control and DMSO as a blank. After 24 hours, Wst-1 reagent was added and incubated for 2 - 4 hours. After that, the CCK-8 reagent was discarded and the absorbance was measured using Tecan Infinite spectrophotometer (λ 450 nm). Calculation of cell survival rate (cell survival rate) is calculated using the formula:

$$SR = \frac{SA-BA}{AN-BA} x100\% \quad (1)$$

Note:
SR = Survival rate
SA = Sample Absorbance
BA = Blank Absorbance
AN = Absorbance Negative Control

Data Analysis

The data displayed is the standard error of the mean (SEM). Data were analyzed GraphPad Prism version 9.0.0. One-way analysis of variance (ANOVA) followed by Tukey's follow-up test was used to determine statistical significance. A P value <0.01 was considered significant.

Results And Discussion

In vitro testing was carried out using the WST-8 method. The WST-8 test is included in the colorimetric test for evaluating cell metabolic activity, which can also be used to see the survival rate of the cells being tested (Stockert, Horobin et.al, 2018). In this study, the cell line used was DU145. The DU145 cell line is a human prostate cancer cell line that is often used in research on prostate cancer (Khan et al., 2023; Khelifi et al., 2020; Russell & Kingsley, 2003).

This study used cisplatin as a control because cisplatin is a first-line treatment for prostate cancer that is used together with carboplatin and oxaliplatin (Brown et al., 2019; Zhu et al., 2015). The mechanism of cisplatin is its ability to interfere with cell division and trigger cell death by forming cross-links with the guanine bases of the DNA double helix chain which causes DNA transcription and replication to be disrupted (Dasari & Bernard Tchounwou, 2014).

The survival rate value obtained will then be used to calculate the IC50 value for each sample tested. The test samples used were α-Mangostin and cisplatin. IC50 is a measurement used as the potential of a compound or substance to influence biological activity which can indicate the concentration of the substance needed to have an effect on 50% of the test material (Barba-Ostria et al., 2022; Ogbonna & Özgör, 2021). The IC50 values obtained for each test material can be seen in Table 1.

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Table 1. %survival rate of α-Mangostin and cisplatin

Concentration			%Survival Rate
α-Mangostin			
50	17.83772078	23.23087673	19.30226067
25	12.79814858	14.5033703	11.88947898
12.5	44.7908235	47.30052737	45.1800158
Cisplatin			
20	40.122328	53.8447681	44.455735
4	67.34827	78.204336	70.482875
0.8	60.06379	55.611987	35.616727

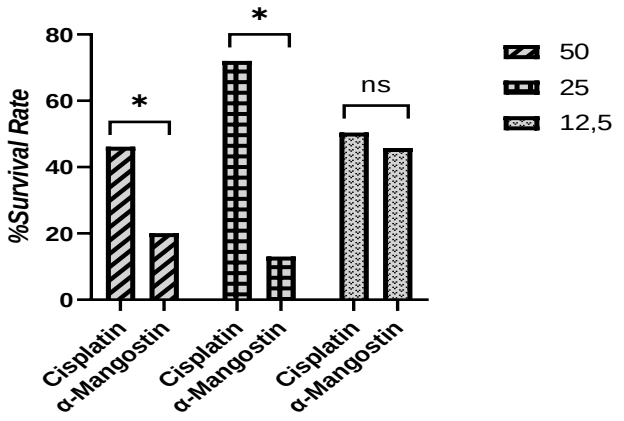

Figure 1. Statistical analysis of survival rate for α-Mangostin and cisplatin. *significantly different, ns is not significantly different

Table 2. IC50 values of α-Mangostin and cisplatin

Compound	IC_{50} (Ppm)	Cytotoxic Activity
α-Mangostin	16.89	Active
Cisplatin	465	Very Active

The survival rate in Table 1 which displays the results of the cytotoxic activity test on DU145 cells shows that the cytotoxic impact increases with increasing concentration. The IC50 value of each test substance is α-Mangostin 16.89 ppm with the active category, cisplatin 4, 65 ppm in the very active category. The survival rate test results showed that at a concentration of 12.5 ppm, there was no significant difference between α-Mangostin and cisplatin.

By enhancing apoptotic activity and preventing proliferation through cell cycle arrest, α-mangostin exhibits anti-breast cancer efficacy in several ways (Nauman & Johnson, 2022). The anti-breast cancer action of α-Mangostin targets inflammatory pathways, mainly inhibiting JNK and p38 of the MAP Kinase pathway, some STAT3 and JAK2 proteins of the JAK-STAT system, and proteins related to the NFκB signaling pathway (Johnson et al., 2012). Throughinhibiting Wnt signaling pathway proteins, α-manganostin has also shown efficacy in preventing breast cancer cells from migrating, invasively settling in new areas, and spreading (Chen et al., 2018). Through various mechanisms, including enhancing apoptotic activity and preventing proliferation through cell cycle arrest, α-

mangostin has anti-breast cancer properties (Nauman & Johnson, 2022). The anti-breast cancer effect of α-Mangostin specifically inhibits inflammatory pathways, including JNK and p38 of the MAP Kinase pathway, proteins related to the NFκB signaling system, and certain proteins STAT3 and JAK2 of the JAK-STAT pathway (Johnson et al., 2012). By blocking Wnt signaling pathway proteins, α-manganostin also demonstrated efficacy in inhibiting migration, invasion, and metastasis of breast cancer cells (Chen et al., 2018).

The cytotoxic test using the WST-8 method is only able to explain the activity in inhibiting cancer cell growth with the IC50 parameter. The IC50 value cannot yet present the molecular mechanism of the sample as an anticancer agent (Gao et al., 2020). Therefore, further research is needed regarding the effects of α-Mangostin as an anticancer (Kim et al., 2020; Meylina et al., 2021; Zhang et al., 2020).

Conclusion

The IC50 values of α-Mangostin and cisplatin against DU 145 cells were 16.89 ppm and 4.65 ppm. At a concentration of 12.5 pmm there was no significant difference between cisplatin and α-Mangostin.

Acknowledgements
Thanks to all parties who have supported the implementation of this research. I hope this research can be useful.

Author's Contribution
Conseptualization: Ajeng Diantini, dan Sriwidodo.; Methodology: Syafika Alaydrus.; Investigasi: Syafika Alaydrus.; Resources : Syafika Alaydrus.; Data Curation : Syafika Alaydrus, Rai Chika Dewi.; Writig-Original draft preparation. Syafika Alaydrus, Rai Chika Dewi.; Writing-Review and Editing: Syafika Alaydrus, Sriwidodo, Rai Chika Dewi.; Visualization: Syafika Alaydrus, Rai Chika Dewi. All authors have read and agreed to the published version of the manuscript.

Funding
Researchers independently funded this research.

Conflicts of Interest
The authors declare no conflict of interest.

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