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Antiproliferative and Apoptosis-Inducing Effects of Iodine Molecule in Differentiated Primary Thyroid Cancer Cells: In Vitro Study

Yulia Kurniawati[1,2], Jihan Atiqah[3], Malinda Meinapuri[4], Daan Khambri[5], Achmad Hussein Sundawa Kartamihardja[6], Aisyah Elliyanti[2]

[1]Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Andalas, Padang, Indonesia
[2]Department of Radiology, Division of Nuclear Medicine and Theranostic Molecular, Faculty of Medicine, Universitas Andalas/Dr M.Djamil Hospital, Padang, Indonesia
[3] Undergraduate Medical Program Study, Faculty of Medicine, Universitas Andalas, Padang, Indonesia
[4]Department of Histology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia
[5]Department of Surgery, Faculty of Medicine, Universitas Andalas/Dr M.Djamil Hospital, Padang, Indonesia.
[6]Department of Nuclear Medicine and Molecular Theranostics, Faculty of Medicine, Universitas Padjadjaran/Hasan Sadikin General Hospital, Bandung, Indonesia

*Corresponding Author: aelliyanti@med.unand.ac.id

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Abstract. Thyroid cancer poses a persistent clinical challenge, particularly when conventional therapies fall short. Growing evidence suggests that molecular iodine (I_2) may exert direct antitumor effects beyond its classical role in thyroid hormone synthesis. This study evaluated the antiproliferative and apoptosis-inducing effects of I_2 on primary cell cultures derived from differentiated thyroid cancer tissue and established dose- and time-response relationships. Primary cultures were exposed to I_2 at concentrations of 5, 10, 20, 40, 80, and 100 µM for 24, 48, and 72 hours. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, with IC_{50} calculated accordingly. Apoptosis was characterized by acridine orange/propidium iodide (AO/PI) double-staining, analyzed by fluorescence microscopy and ImageJ software. I_2 demonstrated clear antiproliferative and apoptotic activity in a dose- and time-dependent manner. The strongest effect appeared at 100 µM after 72 hours, reducing viability to 1.39% ($p < 0.001$). At the IC_{50} of 2.09 µM, iodine significantly suppressed proliferation and induced apoptosis, with an apoptosis rate of 81.77% ± 12.24% ($p = 0.004$). These findings confirm the meaningful antineoplastic potential of I_2 against differentiated thyroid cancer cells. This study supports SDG 3 (Good Health and Well-Being), contributing to efforts to develop more equitable, effective cancer treatment options and to reduce cancer-related morbidity globally.

Keywords: Antiproliferative; Apoptosis; Differentiated Thyroid Cancer; Iodine Molecule; In vitro

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INTRODUCTION

Thyroid cancer is the most prevalent endocrine malignancy originating from the thyroid gland and ranks as the seventh most common cancer site, according to the Global Cancer Observatory (GLOBOCAN) 2022 (Ferlay et al., 2021; Rossi et al., 2021). The incidence of thyroid cancer has been rapidly increasing, with 567.233 new cases reported globally in 2018, representing 3.1% of all malignancies (Bray et al., 2018). Asia has the highest incidence of thyroid cancer in the world, with a mortality rate of approximately 61.3% (Ferlay et al., 2021). In Indonesia, the number of reported thyroid cancer cases rose from 11,470 in 2018 to 13,761 in 2022 (Ferlay et al., 2021; International Agency for Research on Cancer, 2018).

Differentiated thyroid cancer (DTC), which includes papillary thyroid cancer, follicular thyroid cancer, Hurthle cell carcinoma, and poorly differentiated thyroid cancer, arises from follicular cells and constitutes approximately 95% of all thyroid tumors (Mu et al., 2019). Although DTC generally has an excellent long-term prognosis, recurrence occurs in about 35% of patients, with the prevalence of distant metastases varying widely between 5% and 25%, predominantly

affecting the lungs and bones (Nervo et al., 2021). Although disease-specific mortality remains below 5% at ten years, morbidity and mortality rise considerably in recurrent or metastatic disease, underscoring the need for more effective therapeutic strategies (Nervo et al., 2021).

Various therapeutic strategies have been developed to address the increasing burden of cancer cases in Indonesia. Current treatment approaches generally act through multiple biological pathways to eliminate cancer cells, including the inhibition of cell proliferation, induction of apoptosis, and suppression of genetic mutations (Dimarti et al., 2020). Cancer treatment strategies commonly involve a combination of surgery, chemotherapy, and radiotherapy. However, no universally standardized approach has been established as definitively optimal for all cancer types. The adverse effects associated with these therapeutic modalities have driven ongoing research and development of anticancer agents (Dimarti et al., 2020; Ekowati et al., 2017). Previous studies demonstrated that various bioactive compounds, including chemical substances and natural extracts, are capable of inhibiting cell proliferation and inducing apoptosis through modulation of cellular and molecular pathways. These findings highlight the importance of exploring biologically active compounds as potential anticancer agents (Dimarti et al., 2020; Ekowati et al., 2017; Kristiani et al., 2025).

Iodine plays a uniquely central role in thyroid physiology, as the thyroid gland is the primary organ responsible for iodine uptake and metabolism. At the molecular level, high-dose iodine (I_2) has been shown to suppress thyroid hormone synthesis by inhibiting iodide coupling, H_2O_2 formation, cyclic adenosine monophosphate (cAMP) production, and the phospholipase cascade, as well as reducing thyroid-stimulating hormone (TSH) secretion (Nava-Villalba & Aceves, 2014). Additionally, it has been reported to indirectly inhibit the expression and secretion of Vascular endothelial growth factor (VEGF) in thyrocyte cells (Nava-Villalba & Aceves, 2014). The iodide inhibitory effect is reported to be growth-inhibitory, reduce cell density (antiproliferative), and induce apoptosis in thyroid cells. Some studies have demonstrated that high doses of iodine can significantly inhibit cell growth and induce apoptosis (Xiang et al., 2015).

Beyond the thyroid, iodine has demonstrated antineoplastic properties across multiple cancer types. In vitro studies on breast and prostate cancer cell lines have consistently shown that iodine reduces cell viability and enhances apoptosis in a dose-dependent manner (Aranda et al., 2013; Elliyanti et al., 2020). These findings collectively support the hypothesis that iodine may exert broad antiproliferative effects through mechanisms that are not exclusively organ-specific.

Given the established role of iodine in thyroid biology and accumulating evidence of its antineoplastic activity, this study aims to evaluate the antiproliferative and apoptosis-inducing effects of molecular iodine on primary cell cultures derived from differentiated thyroid cancer tissue, with the goal of exploring its potential as an alternative or adjunct therapeutic agent.

METHODS

Study design

This study employed a true experimental in vitro design with a control group using a dose- and time-dependent treatment model, evaluating the effects of escalating iodine molecule concentrations (5–100 µM) on cell viability and apoptotic activity across multiple time points (24, 48, and 72 hours), with untreated cells serving as the control group. The biological material consisted of primary thyroid cancer cells obtained from a patient diagnosed with papillary thyroid carcinoma who underwent total thyroidectomy. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Andalas (Approval No. 251/UN.16.2/KEP-FK/2023). In this in vitro setting, the unit of analysis was the cultured cells rather than the individual donor. All experiments were conducted in triplicate to ensure reproducibility.

Cell Culture

The primary cell culture was cultured at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. Subculturing was then performed to obtain cells in the logarithmic phase. The cells were cultured in *Dulbecco's Modified Eagle Medium* (DMEM) medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin at 37°C in a 5% carbon dioxide (CO_2) environment.

Iodine Molecule Treatment

The cells were plated in a 96-well plate at a density of 2×10^4 cells/well and incubated for 24 hours, then treated with 5 µM, 10 µM, 20 µM, 40 µM, 80 µM, and 100 µM of iodine molecule. The treatments were done for 24, 48, and 72 hours. The

experiments were repeated in triplicate. The control cells were treated with medium only.

Cell Viability

Cell viability was determined by MTT [3-(4 - 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay in triplicate. At the end of incubation, the medium was removed, MTT (5 mg/ml) was added to the wells, and the plate was incubated for 4 hours at 37°C. The supernatants were removed, 100 µL of dimethyl sulfoxide (DMSO) was added, and the absorbance was measured at 550 nm using a colorimeter. The cell viability in percentage (%) was calculated as the optical density (OD) of the tested culture divided by the OD of the control, multiplied by 100%. A regression analysis was performed to determine the half-maximal inhibitory concentrations of cell growth (IC_{50}) (Cancer Chemoprevention Research Center Fakultas Farmasi UGM, 2013; Kamiloglu et al., 2020).

Cell Apoptosis

Double staining with acridine orange (AO) and propidium iodide (PI) helps differentiate between viable and non-viable cells. After incubating the cells for 24 hours, the medium was replaced with an IC_{50} dose of iodine molecules, followed by an additional incubation period of 24, 48, and 72 hours. AO/PI were then added to each well after two washes with PBS. The cells were observed with a fluorescence microscope, and images were captured for further analysis. The fluorescent images were subsequently processed using ImageJ software to quantify the percentage of apoptotic cells (Ibrahim et al., 2021; Sulaiman et al., 2020).

Data Analysis

All experiments were conducted in triplicate. Bivariate data were analyzed using one-way ANOVA followed by the Bonferroni Post Hoc test. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Cell viability

The effect of iodine molecules at various concentrations (5 µM, 10 µM, 20 µM, 40 µM, 80 µM, 100 µM) on the viability of primary cell cultures over treatment durations of 24, 48, and 72 hours is presented in Table 1. Iodine significantly reduced cell proliferation in a dose- and time-dependent manner, as shown in Table 1. A significant decrease in cell proliferation was observed after treatment with 100 µM at 72 hours, with cell viability reduced to 1.39% (*$p < 0.001$ vs untreated control*). Iodine treatment for 72 hours significantly reduced thyroid cancer cell viability in a dose-dependent manner compared to the untreated control, as shown in Table 2.

Table 1. Effect of iodine molecule on cell viability

Iodine Concentration	Cell Viability (%)			p
	24 hours	48 hours	72 hours	
5 µM	99.97	89.31	21.61	<0.001
10 µM	96.00	76.28	19.25	<0.001
20 µM	90.15	70.94	15.56	<0.001
40 µM	86.91	63.23	7.52	<0.001
80 µM	81.34	44.04	4.27	<0.001
100 µM	72.54	27.26	1.39	<0.001
0 µM (Control)	100.00	100.00	100.00	
p	<0.001	<0.001	<0.001	

Table 2. Effect of iodine concentration on cell viability at 72 hours of treatment compared to the untreated control

Iodine Concentration	Cell Viability Means ± SD (%)	p
5 µM	21.61 ± 1.29	<0.001
10 µM	19.25 ± 2,62	0.001
20 µM	15.56 ± 1.09	<0.001
40 µM	7.52 ± 2.02	0.001
80 µM	4.27 ± 2.36	0.001
100 µM	1.39 ± 0.24	<0.001
0 µM (Control)	100.00 ± 0.00	

The IC_{50} values for the iodine molecule were determined at 24-, 48-, and 72-hour intervals using linear regression analysis of dose response curves, as shown in Table 3. The observed IC_{50} values at these time points were 196.04 µM, 63.36 µM, and 2.09 µM, respectively. The treatment with the iodine molecule at 72 hours showed the most significant effect, indicating the highest cytotoxic potency, with an IC_{50} value of 2.09 µM.

Table 3. Linear regression equation of cell viability and IC50

Duration of Therapy (hours)	Linear Regression Equation	R^2	IC_{50} (µM)
24	Y = -0.2463x + 98.285	0.9395	196.04
48	Y= -0.5676x + 85.966	0.9673	63.36
72	Y= -3.4677x + 57.264	0.9994	2.09

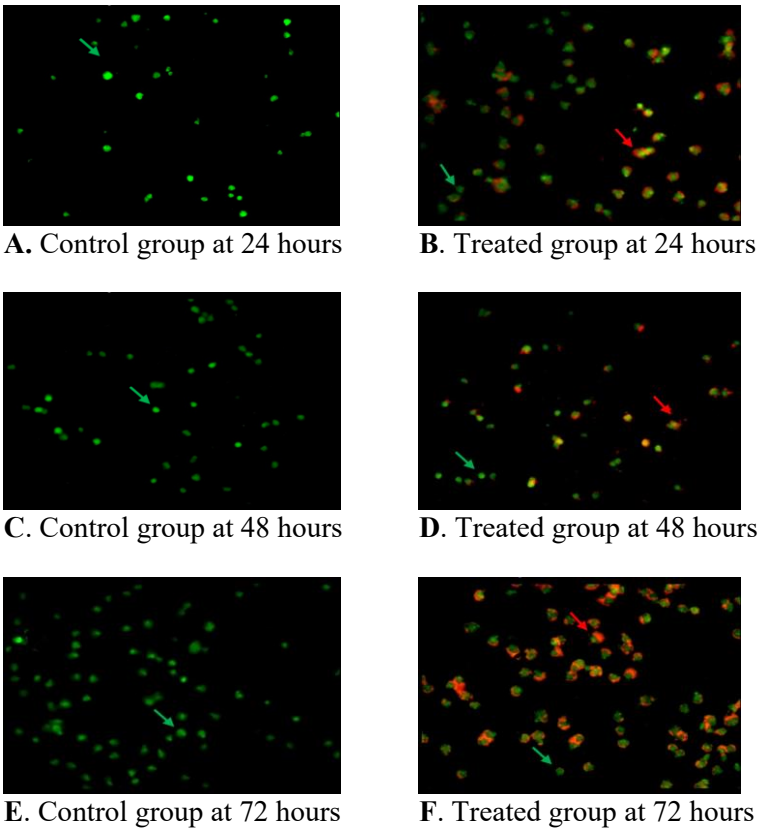
Cell apoptosis with IC_{50}

The AO/PI double-staining assay evaluated apoptosis at a concentration of 2.09 µM, which was identified as the optimal IC_{50} value from prior analyses. Quantitative analysis was conducted based on fluorescence differences using ImageJ software. The highest percentage of apoptosis was observed after 72 hours of treatment, with up to 81.76% of cells undergoing apoptosis. This was followed by the 48-hour treatment period, during which 52.95 % of the cells exhibited apoptosis, and by a 24-hour treatment period, during which 34.99% of the cells exhibited apoptosis, as shown in Table 4, which identified a statistically significant relationship ($p = 0.004$). Post hoc analysis revealed significant differences in the percentage of apoptotic cells between the 72-hour group (81.77% ± 12.24) and both the 24-hour (34.99% ±2.31) and 48-hour groups (52.95%±12.33).

Table 4. The Effect of the iodine molecule on the percentage of apoptotic cells

Duration of Therapy Group	Cell Apoptosis (%) ± SD	p
Control	0	0.004
24 hours	34.99 ± 2.31	
48 hours	52.95 ± 12.33	
72 hours	81.77 ± 12.24	

Green fluorescence (indicated by green arrows) represents viable cells, while red/orange fluorescence (indicated by red arrows) represents apoptotic or non-viable cells. A progressive increase in apoptotic cells is observed in treated groups, particularly at 72 hours, as shown in Figure 1.



A. Control group at 24 hours
B. Treated group at 24 hours
C. Control group at 48 hours
D. Treated group at 48 hours
E. Control group at 72 hours
F. Treated group at 72 hours

Figure 1. Fluorescence microscopy images of primary thyroid cancer cells stained with acridine orange/ propidium iodide (AO/PI), comparing control and iodine-treated groups at 24, 48, and 72 hours. (A, C, E) Control groups at 24, 48, and 72 hours, respectively, showed predominantly viable cells. (B, D, F) Treated groups at corresponding time points, demonstrating increased apoptotic cell populations over time.

The effects of the iodine molecule as both antiproliferative and apoptosis inducers in differentiated primary thyroid cancer cells demonstrated a significant dose- and time-dependent reduction in cell viability ($p<0.05$). After treatment with 100 µM for 72 hours, we observed a substantial decrease in cell proliferation, with a reduction in cell viability to 1.39% (*$p < 0.001$ vs untreated control*). These findings are consistent with recent literature showing that iodine-related interventions can modulate papillary/differentiated thyroid cancer cell behavior through proliferation-, apoptosis-, and migration-related pathways (X. Zhang et al., 2022).

Therapeutic iodide concentration (> 1 µM) exerted immediate effects and rapidly suppressed thyroid function by blocking iodide oxidation (Wolff-Chaikoff effect) (Panneels et al., 2009). The increasing effect might correlate with vascular endothelial growth factor-A (VEGF-A), which promotes angiogenesis and supports cancer cell growth. Further studies are needed to assess the role of VEGF-A. In contrast, the antiproliferative effect of high-concentration iodine, also called iodide inhibition, was mediated by a block of thyroid signaling, including hydrogen peroxide (H2O2) formation inhibition, cyclic adenosine monophosphate (cAMP) formation inhibition, thyroid hormone inhibition, and block of thyroid vascularization (Milanesi & Brent, 2017; Pirahanci et al., 2025; Yang & Cao, 2022). The findings of this study indicate that the Iodine molecule at a concentration of 5 µM is inhibitory when the therapy duration is 72 hours. In contrast, a concentration of 10 µM iodine exhibited a greater inhibitory effect over the exact duration of therapy.

This result is similar to several previous studies (Gärtner et al., 2010; Liu et al., 2010). Liu et al. (2010) found that iodine inhibited thyroid cancer cell proliferation in a dose- and time-dependent manner, using concentrations 25, 50, 75, and 100 µM over 24, 48, and 72 hours of therapy. On the other hand, Gärtner et al. (2010) observed that 24-hour therapy with 10 µM iodine already had an inhibitory effect, though not significant, whereas 100 µM and 500 µM showed a significant decrease in cell viability compared with controls. However, it has different results from the study conducted by Zhang et al, which showed that only the dose concentration had an inhibition effect, but not the duration of therapy (D. Zhang et al., 2019). Although no previous studies have used the same concentration as in our study, the consistency of our findings with prior work strengthens the evidence that iodine exerts antiproliferative and apoptosis-inducing effects in thyroid cancer.

The optimal IC_{50} was observed at 72 hours (2.09 µM), indicating the highest cytotoxic potency of the iodine molecule and higher effectiveness. Previous studies have not reported the IC_{50} value of iodine for thyroid cancer cells (Gärtner et al., 2010; Liu et al., 2010). The study on breast cancer cells (MCF-7) found the IC_{50} value of iodine was 204.7 µM with a 48-hour duration of therapy (Mendieta et al., 2019). In addition, a study on prostate cancer cells (LNCaP) found the IC_{50} value of iodine was 181 µM with a 72-hour duration of therapy (Aranda et al., 2013). The IC_{50} in our study was lower than that reported in the mentioned studies. The thyroid is the primary organ that expresses sodium/iodide Symporter (NIS) mRNA, which is crucial for iodide uptake. Most thyroid cancer cells retain functional NIS expression (Kogai & Brent, 2013; Micali et al., 2014).

On the other hand, it has been known that NIS is expressed in extrathyroidal tissues, including breast and prostate cancer (Navarra et al., 2010; Olvera-Caltzontzin et al., 2013). Although the sodium/iodide symporter (NIS) is also expressed in breast and prostate cancer cells, its expression level is lower than in the thyroid (Kogai & Brent, 2013; Micali et al., 2014). The thyroid NIS shows the highest expression, predominantly localized to the basolateral membrane, whereas in breast and prostate cancers, it is localized to the cytoplasm or perinuclear region (Kogai & Brent, 2013; Micali et al., 2014; Olvera-Caltzontzin et al., 2013).

This study also demonstrated that the highest percentage of apoptosis was observed at 72 hours of treatment, with up to 81.77 % of cells undergoing apoptosis. The iodine molecule significantly induced apoptosis, with the percentage of apoptosis increasing over the duration of therapy, as shown in Table 4. The apoptosis-inducing effect of iodine in thyroid cancer occurs through the mitochondrial pathway, where iodine disrupts mitochondrial membrane potential, increases the levels of mitochondria-related pro-apoptotic molecules, and promotes the release of cytochrome C from mitochondria (Liu et al., 2010). Liu et al. (2010) reported that iodine promotes apoptosis in thyroid cancer cells by modulating the MAPK-related signaling pathway, and is involved in the downregulation of p53 and upregulation of p21 and Bcl-xL expression.

Similarly, Gartner et al. demonstrated that

molecular iodine induced apoptosis in malignant thyroid epithelial cells by directly affecting mitochondria (Gärtner et al., 2010). The mechanism involved a decrease in mitochondrial membrane potential, the initial stage of intrinsic apoptosis (Gärtner et al., 2010). These effects demonstrate that iodine, a naturally occurring molecule accumulated by thyroid cells via the NIS transporter, is not only antiproliferative but also induces apoptosis, making it relevant for the development of adjuvant therapies for differentiated thyroid cancer.

The novelty of this study lies in the use of primary cell cultures derived from differentiated thyroid cancer tissue to investigate the dose- and time-dependent effects of molecular iodine (I_2), offering a more clinically relevant model and providing new insights into its potential as an alternative therapeutic agent. This study supports SDG 3 (Good Health and Well-Being) and SDG 9 (Industry, Innovation and Infrastructure) by contributing to evidence-based cancer research, advancing the development of more effective therapeutic strategies, and promoting laboratory-based innovation that may improve future thyroid cancer management.

CONCLUSION

The iodine molecule reduced cell proliferation and induced apoptosis in differentiated primary thyroid cancer cells, effects that were dose- and duration-dependent. This suggests that iodine has potential as a therapeutic enhancer in the management of differentiated thyroid cancer. However, further studies, including an in vivo study, are needed to evaluate how iodine affects tumor microenvironments. Future studies should also examine the molecular mechanism of iodine action, its effect in combination with standard therapy, and its activity in multiple patient-derived samples.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. YK, JA, and AE conceived and designed the study, performed data analysis, and drafted the original manuscript. MM contributed to the study methodology. DK and AHSK provided supervision throughout the research process. All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

REFERENCES

Aranda, N., Sosa, S., Delgado, G., Aceves, C., & Anguiano, B. (2013). Uptake and antitumoral effects of iodine and 6-iodolactone in differentiated and undifferentiated human prostate cancer cell lines. *Prostate*, *73*(1), 31–41. https://doi.org/10.1002/pros.22536

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424. https://doi.org/10.3322/caac.21492

Cancer Chemoprevention Research Center Fakultas Farmasi Universitas Gajah Mada. (2013). *Protokol uji sitotoksik metode MTT*.

Dimarti, S. C., Susilaningsih, N., & Yuniati, R. (2020). Phycocyanin from Spirulina platensis Induces Cytotoxicity and Apoptosis in T47D Cells. *Biosaintifika*, *12*(1), 28–34. https://doi.org/10.15294/biosaintifika.v12i1.22992

Ekowati, N., Mumpuni, A., & Muljowati, J. S. (2017). Effectiveness of Pleurotus ostreatus Extract Through Cytotoxic Test and Apoptosis Mechanism of Cervical Cancer Cells. *Biosaintifika*, *9*(1), 148. https://doi.org/10.15294/biosaintifika.v9i1.7546

Elliyanti, A., Susilo, V. Y., Setiyowati, S., & Rao, P. V. (2020). An iodine treatments effect on cell

proliferation rates of breast cancer cell lines; in vitro study. *Open Access Macedonian Journal of Medical Sciences*, *8*(B), 1064–1070. https://doi.org/10.3889/oamjms.2020.5447

Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D. M., Piñeros, M., Znaor, A., & Bray, F. (2021). Cancer statistics for the year 2020: An overview. *International Journal of Cancer*, *149*(4), 778–789. https://doi.org/10.1002/ijc.33588

Gärtner, R., Rank, P., & Ander, B. (2010). The role of iodine and δ-iodolactone in growth and apoptosis of malignant thyroid epithelial cells and breast cancer cells. *Hormones*, *9*(1), 60–66. https://doi.org/10.14310/horm.2002.1254

Ibrahim, A. A., Kareem, M. M., Al-Noor, T. H., Al-Muhimeed, T., Alobaid, A. A., Albukhaty, S., Sulaiman, G. M., Jabir, M., Taqi, Z. J., & Sahib, U. I. (2021). Pt(Ii)-thiocarbohydrazone complex as cytotoxic agent and apoptosis inducer in caov-3 and ht-29 cells through the p53 and caspase-8 pathways. *Pharmaceuticals*, *14*(6). https://doi.org/10.3390/ph14060509

International Agency for Research on Cancer. (2018). *Global Cancer Observatory: Cancer Today - Indonesia*. World Health Organization.

Kamiloglu, S., Sari, G., Ozdal, T., & Capanoglu, E. (2020). Guidelines for cell viability assays. *Food Frontiers*, *1*(3), 332–349. https://doi.org/10.1002/fft2.44

Kogai, T., & Brent, G. a. (2013). to Targeting for Cancer Therapeutics. *Pharmacology & Therapeutics*, *135*(3), 355–370. https://doi.org/10.1016/j.pharmthera.2012.06.007.The

Kristiani, E. B. E., Kasmiyati, S., Martono, Y., & Kachingwe, B. H. (2025). Extract Formulation of Zingiber officinale and Phylanthus reticulatus Induces Apoptosis of WiDr Colon Cancer Cells. *Biosaintifika*, *17*(3), 370–381. https://doi.org/10.15294/biosaintifika.v17i3.25776

Liu, X. H., Chen, G. G., Vlantis, A. C., Tse, G. M., & van Hasselt, C. A. (2010). Iodine induces apoptosis via regulating MAPKs-related p53, p21, and Bcl-xL in thyroid cancer cells. *Molecular and Cellular Endocrinology*, *320*(1–2), 128–135. https://doi.org/10.1016/j.mce.2010.02.004

Mendieta, I., Nuñez-Anita, R. E., Nava-Villalba, M., Zambrano-Estrada, X., Delgado-González, E., Anguiano, B., & Aceves, C. (2019). Molecular iodine exerts antineoplastic effects by diminishing proliferation and invasive potential and activating the immune response in mammary cancer xenografts. *BMC Cancer*, *19*(1), 1–12. https://doi.org/10.1186/s12885-019-5437-3

Micali, S., Bulotta, S., Puppin, C., Territo, A., Navarra, M., Bianchi, G., Damante, G., Filetti, S., & Russo, D. (2014). Sodium iodide symporter (NIS) in extrathyroidal malignancies: Focus on breast and urological cancer. *BMC Cancer*, *14*(1), 1–12. https://doi.org/10.1186/1471-2407-14-303

Milanesi, A., & Brent, G. A. (2017). Iodine and Thyroid Hormone Synthesis, Metabolism, and Action. In *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals* (Vol. 1896). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802168-2.00012-9

Mu, Z.-Z., Zhang, X., & Lin, Y.-S. (2019). Identification of Radioactive Iodine Refractory Differentiated Thyroid Cancer. *Chonnam Medical Journal*, *55*(3), 127. https://doi.org/10.4068/cmj.2019.55.3.127

Nava-Villalba, M., & Aceves, C. (2014). 6-Iodolactone, key mediator of antitumoral properties of iodine. *Prostaglandins and Other Lipid Mediators*, *112*, 27–33. https://doi.org/10.1016/j.prostaglandins.2014.07.001

Navarra, M., Micali, S., Lepore, S. M., Cesinaro, A. M., Celano, M., Sighinolfi, M. C., De Gaetani, C., Filetti, S., Bianchi, G., & Russo, D. (2010). Expression of the Sodium/Iodide Symporter in Human Prostate Adenocarcinoma. *Urology*, *75*(4), 773–778. https://doi.org/10.1016/j.urology.2009.10.011

Nervo, A., Ragni, A., Retta, F., Gallo, M., Piovesan, A., Liberini, V., Gatti, M., Ricardi, U., Deandreis, D., & Arvat, E. (2021). Bone metastases from differentiated thyroid carcinoma: current knowledge and open issues. *Journal of Endocrinological Investigation*, *44*(3), 403–419. https://doi.org/10.1007/s40618-020-01374-7

Olvera-Caltzontzin, P., Delgado, G., Aceves, C., & Anguiano, B. (2013). Iodine uptake and prostate cancer in the TRAMP mouse model. *Molecular Medicine*, *19*(1), 409–416. https://doi.org/10.2119/molmed.2013.00093

Panneels, V., Juvenal, G., Boeynaems, J. M., Dumont, J. E., & van Sande, J. (2009). Iodide Effects on the Thyroid: Biochemical, Physiological, Pharmacological, and Clinical Effects of Iodide in the Thyroid. In *Comprehensive Handbook of Iodine: Nutritional, Biochemical, Pathological and Therapeutic Aspects*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-374135-6.00032-7

Pirahanci, Y., Toro, F., & Jialal, I. (2025).

Physiology, thyroid stimulating hormone. In *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK499850/%0A

Rossi, E. D., Pantanowitz, L., & Hornick, J. L. (2021). A worldwide journey of thyroid cancer incidence centred on tumour histology. *The Lancet Diabetes & Endocrinology*, *9*(4), 193–194. https://doi.org/10.1016/S2213-8587(21)00049-8

Sulaiman, G. M., Waheeb, H. M., Jabir, M. S., Khazaal, S. H., Dewir, Y. H., & Naidoo, Y. (2020). Hesperidin Loaded on Gold Nanoparticles as a Drug Delivery System for a Successful Biocompatible, Anti-Cancer, Anti-Inflammatory and Phagocytosis Inducer Model. *Scientific Reports*, *10*(1), 1–16. https://doi.org/10.1038/s41598-020-66419-6

Xiang, J., Wang, X., Wang, Z., Wu, Y., Li, D., Shen, Q., Sun, T., Guan, Q., & Wang, Y. (2015). Effect of Different Iodine Concentrations on Well-Differentiated Thyroid Cancer Cell Behavior and its Inner Mechanism. *Cell Biochemistry and Biophysics*, *71*(1), 299–305. https://doi.org/10.1007/s12013-014-0198-8

Yang, Y., & Cao, Y. (2022). The impact of VEGF on cancer metastasis and systemic disease. *Seminars in Cancer Biology*, *86*(P3), 251–261. https://doi.org/10.1016/j.semcancer.2022.03.011

Zhang, D., Xu, X., Li, J., Yang, X., Sun, J., Wu, Y., & Qiao, H. (2019). High iodine effects on the proliferation, apoptosis, and migration of papillary thyroid carcinoma cells as a result of autophagy induced by BRAF kinase. *Biomedicine and Pharmacotherapy*, *120*(September), 109476. https://doi.org/10.1016/j.biopha.2019.109476

Zhang, X., Zhang, F., Li, Q., Feng, C., & Teng, W. (2022). Iodine nutrition and papillary thyroid cancer. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1022650