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Original Article

Anticancer Potential of Didymin by Inducing Apoptosis in Thyroid Cancer TPC-1 Cells

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ABSTRACT

Background: Thyroid cancer, a tumor arising from the thyroid gland, impacts a considerable portion of the population, with incidence rates exhibiting an upward trend globally. The mortality rates for thyroid cancer are rather low; yet, the increasing occurrence demands a comprehensive study of its epidemiology. **Objectives:** The present study assessed the efficacy of didymin in efficiently eliminating thyroid cancer cells by inhibiting viability and enhancing apoptosis, thereby hindering the growth of thyroid cancer cells. **Materials and Methods:** The cytotoxic levels of didymin against thyroid cancer TPC-1 cells were evaluated. The Lactate Dehydrogenase (LDH) release assay was conducted using kits. Apoptotic cell death levels in the didymin-treated TPC-1 cells were evaluated using a dual staining technique. The caspase-3, -8, and -9 enzyme activities in the cells were evaluated using test kits. **Results:** The treatment of various concentrations of didymin significantly reduced the viability and increased the LDH activity in the thyroid cancer TPC-1 cells in a dose-dependent manner. The didymin treatment at 20 µM concentration markedly enhanced apoptosis in TPC-1 cells. In addition, didymin treatment also markedly enhanced the caspase-3, -8, and -9 activities in the TPC-1 cells. **Conclusion:** In conclusion, our present research demonstrates didymin as a promising antitumor agent for thyroid cancer *in vitro* by reducing cell growth and promoting caspase-mediated apoptosis in TPC-1 cells. These results highlight that didymin can be a hopeful option to treat thyroid cancer.

Keywords: TPC-1 cell line, Lactate dehydrogenase, Didymin, Apoptosis, Thyroid cancer.

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INTRODUCTION

Thyroid cancer, a malignancy developing from the thyroid gland, affects a significant portion of the population, with incidence rates demonstrating an increasing trend worldwide. The increase in incidence is especially pronounced in middle- and high-income areas due to urbanization and economic growth. Globally, thyroid cancer accounts for nearly 1% of all newly diagnosed cancer incidences, establishing it as the most widespread endocrine malignancy.[1] Thyroid cancer predominantly develops from follicular epithelial cells or parafollicular C cells; the former are further categorized into papillary thyroid carcinoma, constituting 80-85% of incidences, and follicular thyroid cancer, constituting 10-15% of incidences. Medullary thyroid carcinoma represents about 5% of cases, while anaplastic thyroid carcinoma accounts for less than 5%.[2] Papillary thyroid cancer is the widespread, with an incidence that has elevated significantly over the last years, representing approximately 80% of all thyroid cancer incidences. It is more common in women than in men, and usually presents between the ages of 50-60s of life. Mortality rates for thyroid cancer are relatively low, yet the rising incidence necessitates a thorough understanding of its epidemiology.[3]

The pathophysiology of thyroid cancer is multifaceted, involving a complex interplay of genetic mutations, epigenetic changes, and environmental causes that disrupt normal thyroid cell development. Genetic mutations frequently participate in the onset of papillary and follicular thyroid cancers. These mutations can activate intracellular signaling cascades, resulting in uncontrolled cell growth, reduced apoptosis, and enhanced angiogenesis.[4] BRAF mutations are widely found in papillary thyroid cancer and are connected with aggressive tumor behavior and poor clinical outcomes. The tumor microenvironment also plays a critical role in thyroid cancer development. These molecular aberrations disrupt the normal function of thyroid cells, resulting in uncontrolled growth and the initiation of thyroid cancer.[5]

Current treatment modalities for thyroid cancer primarily involve surgical removal, radioactive iodine therapy, and suppression of thyroid hormone. Total thyroidectomy, often followed by radioactive iodine ablation, has long served as the

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standard approach for managing most stages of differentiated thyroid cancer. However, challenges persist in treating advanced or recurrent disease, as well as radioiodine-refractory cases, and can sometimes be aggressive and lethal.[6] External beam radiation therapy is a standard treatment for a variety of cancers and is commonly used to treat thyroid cancers. The utilization of adjuvant radioactive iodine treatment aims to eliminate residual thyroid tissue and microscopic disease, but its effectiveness diminishes in patients with distant metastases or aggressive tumor histologies.[7] The therapeutic landscape for advanced thyroid cancer has evolved with the introduction of targeted therapies, notably kinase inhibitors, which have demonstrated efficacy in patients with radioiodine-refractory differentiated thyroid carcinoma, medullary thyroid carcinoma, and anaplastic thyroid carcinoma. Despite these advances, challenges remain in identifying the optimal sequencing and combination strategies for targeted therapies, as well as in managing treatment-related toxicities.[8]

Despite the overall favorable prognosis associated with differentiated thyroid cancer, a subset of patients experience disease recurrence or progression, underscoring the demand for more potential and personalized therapeutic strategies.[9] Furthermore, the increasing cases of thyroid cancer and the potential for long-term complications associated with conventional treatments highlight the importance of exploring safe and alternative treatment options. The limitations of current treatment modalities and the potential for long-term complications underscore the need for safe and effective alternative treatment options that can enhance patient outcomes and quality of life.[10] Didymin, a isosakuranetin 7-O-rutinoside, is a bioactive flavonoid glycoside compound present in numerous citrus fruits, including oranges, lemons, mandarins, bergamots, grapefruits, and chachi fruit. It has already been reported that didymin demonstrated various biological functions, including hepatoprotective,[11] cardioprotective,[12] antidiabetic,[13] and neuroprotective[14] activities. Furthermore, didymin effectively induced apoptosis and showed antitumor activity against human lung cancer,[15] neuroblastoma,[16] and hepatoma[17] cells. Nonetheless, its anticancer efficacy in thyroid cancer remains predominantly unknown. This study indicated that didymin can efficiently eliminate thyroid cancer cells by inhibiting viability and enhancing apoptosis, hence hindering the growth of thyroid cancer cells.

MATERIALS AND METHODS

Cell culture

The human papillary thyroid cancer TPC-1 cells were purchased from ATCC, USA. The cells were then cultivated in DMEM enriched with 10% FBS and 1% antimycotic cocktail under a 5% CO_2 incubator. Upon reaching 80% confluency, cells were harvested and utilized for further tests.

MTT cytotoxicity assay

The MTT cytotoxicity test was used to evaluate the efficacy of didymin on the growth of TPC-1 cells.[18] Cells were cultured on a 96-well plate for 24 hr at a 5×10^3 density cells/well. Subsequently, cells were exposed to didymin (1, 5, 10, 20, 40, 80, and 100 µM) for 24 hr. Subsequent to treatment, 20 µL of MTT and 100 µL of DMEM were mixed to each well and incubated for 4 hr. After that, DMSO (100 µL) was mixed to the wells to liquefy the formed formazan deposits, after which a microplate reader was employed to assess the absorbance at 570 nm.

Lactate Dehydrogenase (LDH) release assay

LDH is a soluble enzyme released into the bloodstream during cellular injury and can serve as a signal of cellular toxicity. Consequently, the release of LDH was employed to assess the cytotoxicity of didymin on TPC-1 cells. Cells were cultured for 24 hr with different doses of didymin (1-100 µM). The LDH activity was quantified with the LDH Cytotoxicity Assay Kit (Elabscience, USA) in accordance with the manufacturer's specifications, and the absorbance was recorded at 490 nm.

Dual staining

The apoptotic level in control and didymin-exposed TPC-1 cells were examined using AO/EB staining.[19] Cells were cultivated in a 24-well plate for 24 hr, and subsequently treated with didymin (20 µM) for another 24 hr. Subsequently, AO/EB stains (100 µg/mL) were introduced to each well for 5 min, after which the strength of the resultant fluorescence was evaluated using a fluorescent microscope to identify apoptotic levels in the TPC-1 cells.

Analysis of caspases activity

Commercial diagnostic kits were utilized to analyze the caspase-3, -8, and -9 activities in the untreated and didymin-treated TPC-1 cells. The tests were conducted in accordance with the manufacturer's recommended specifications (Abcam, USA).

Statistical analysis

The results were analyzed using GraphPad Prism software and presented as mean±SD of triplicate assays. The differences in the values of treatment groups was evaluated using one-way ANOVA and Tukey's *post hoc* assay, with significance established at $p<0.05$.

RESULTS

Effect of didymin on the thyroid cancer TPC-1 cell viability

The cytotoxicity of the didymin on TPC-1 cell proliferation was investigated using the MTT assay, with findings depicted in Figure 1. The MTT assay findings demonstrate that treatment with didymin at several concentrations (1-100 µM) significantly reduced TPC-1 cell viability at dose-dependent manner. A significant diminution in the TPC-1 cell growth was seen after

exposure to high dosages of didymin. The IC_{50} value of the didymin was determined to be 20 µM after a 24-hr treatment. Accordingly, 20 µM was utilized for further experiments as the IC_{50} dosage.

Effect of the didymin treatment on the LDH activity in TPC-1 cells

We assessed whether incubation with didymin in TPC-1 cells might elicit cytotoxicity via assessing the LDH activity. For this, TPC-1 cells were exposed to didymin for 24 hr, and the LDH release level was assessed using an LDH assay kit. Figure 2 demonstrated that didymin treatment induced considerable cytotoxicity in TPC-1 cells, which is verified by the increased LDH activity.

Effect of didymin treatment on the apoptosis in TPC-1 cells

Figure 3 illustrates the results of the dual staining assay, which validates the apoptotic effects of didymin on TPC-1 cells. The results demonstrate that didymin treatment at 20 µM resulted in a higher number of red fluorescence cells compared with control cells. The green fluorescent cells in the control group signify the existence of normal live cells, but the increased number of red fluorescent cells in the didymin-treated cells suggests a greater occurrence of apoptotic cells. These results are also supported by the results of DOX treatment.

Effect of didymin on the caspase activities in the TPC-1 cells

The concentrations of caspase-3, -8, and -9 were assessed in the untreated and didymin-treated TPC-1 cells, with the findings illustrated in Figure 4. The control cell exhibited reduced normal caspase-3/8/9 activities. However, the treatment with 20 µM of didymin dramatically enhanced the caspase-3/8/9 activities in thyroid cancer TPC-1 cells. The DOX treatment also reduced the caspase enzyme activities in the TPC-1 cells, which further supports the activity of didymin.

DISCUSSION

Thyroid cancer, despite its relative rarity, garners significant attention due to its diverse morphological and clinical presentations, ranging from indolent papillary carcinomas to highly aggressive anaplastic carcinomas. The rising incidence of thyroid cancer, especially among women worldwide, further underscores the importance of understanding this malignancy. The implication of thyroid cancer extends beyond its mere presence, impacting patients' lives through treatment-related morbidities, psychological distress, and economic burdens.[20] Advanced thyroid cancer can significantly reduce long-term survival rates, particularly when the tumor extends beyond the thyroid gland, affecting nearby structures. The pathophysiology of thyroid cancer comprises a complex interplay of genetic alterations, epigenetic modifications, and environmental causes that disrupt normal thyroid cell growth. Genetic mutations play an essential role in the initiation of thyroid cancer, with specific mutations associated with distinct histological subtypes and clinical behaviors.[21] The implications of understanding the molecular pathophysiology of thyroid cancer are profound, as they pave the way for the advancement of targeted treatments that particularly address the underlying genetic and molecular drivers of the disease. Conventional treatments for thyroid cancer can be effective in many cases, but they are also associated with potential adverse effects. The side effects of traditional treatments and the existence of aggressive forms of thyroid cancer highlight the imperative for ongoing research into safer and more effective therapeutic options.[22]

Apoptosis is a tightly regulated type of programmed cell death, plays a pivotal role in regulating tissue homeostasis and preventing uncontrolled cell proliferation. The equilibrium between cell death and proliferation is crucial for the proper

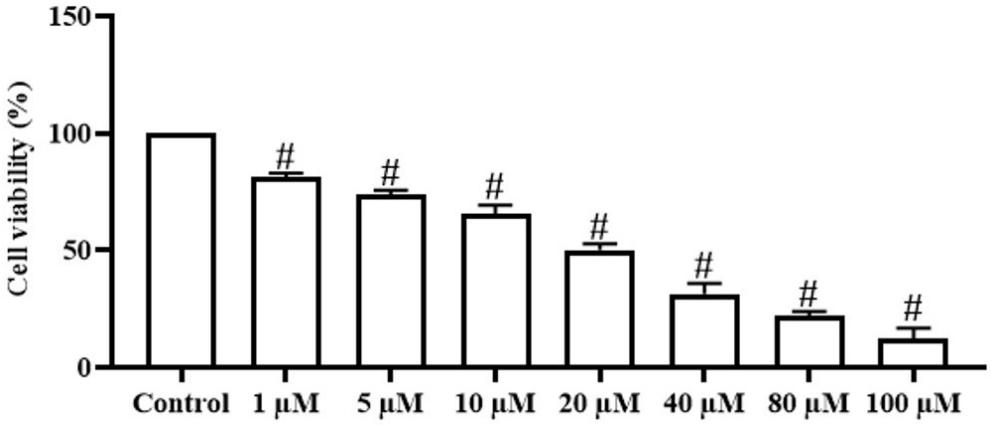

Figure 1: Effect of didymin on the thyroid cancer TPC-1 cell viability. The administration of 1-100 µM of didymin significantly reduced the viability of thyroid cancer TPC-1 cells at dose-dependent manner. The IC_{50} dosage of didymin for TPC-1 cells was determined to be 20 µM. Values are presented as Mean±SD from triplicate experiments. The results were statistically analysed using one-way ANOVA and Tukey's *post hoc* tests. '#' indicates that values significantly vary from the control group at $p<0.05$.

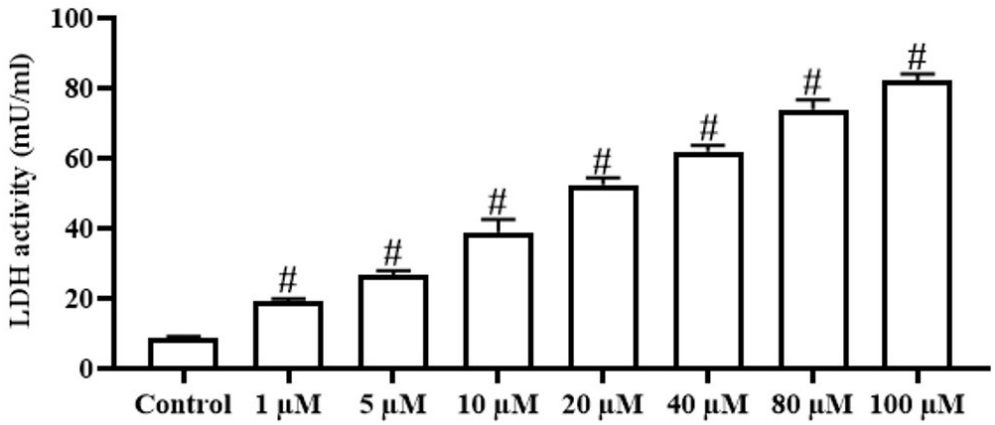

Figure 2: Effect of the didymin treatment on the LDH activity in TPC-1 cells. Values are presented as mean±SD from triplicate experiments. The results were statistically analysed using one-way ANOVA and Tukey's *post hoc* tests. '#' indicates that values significantly vary from the control group at $p<0.05$.

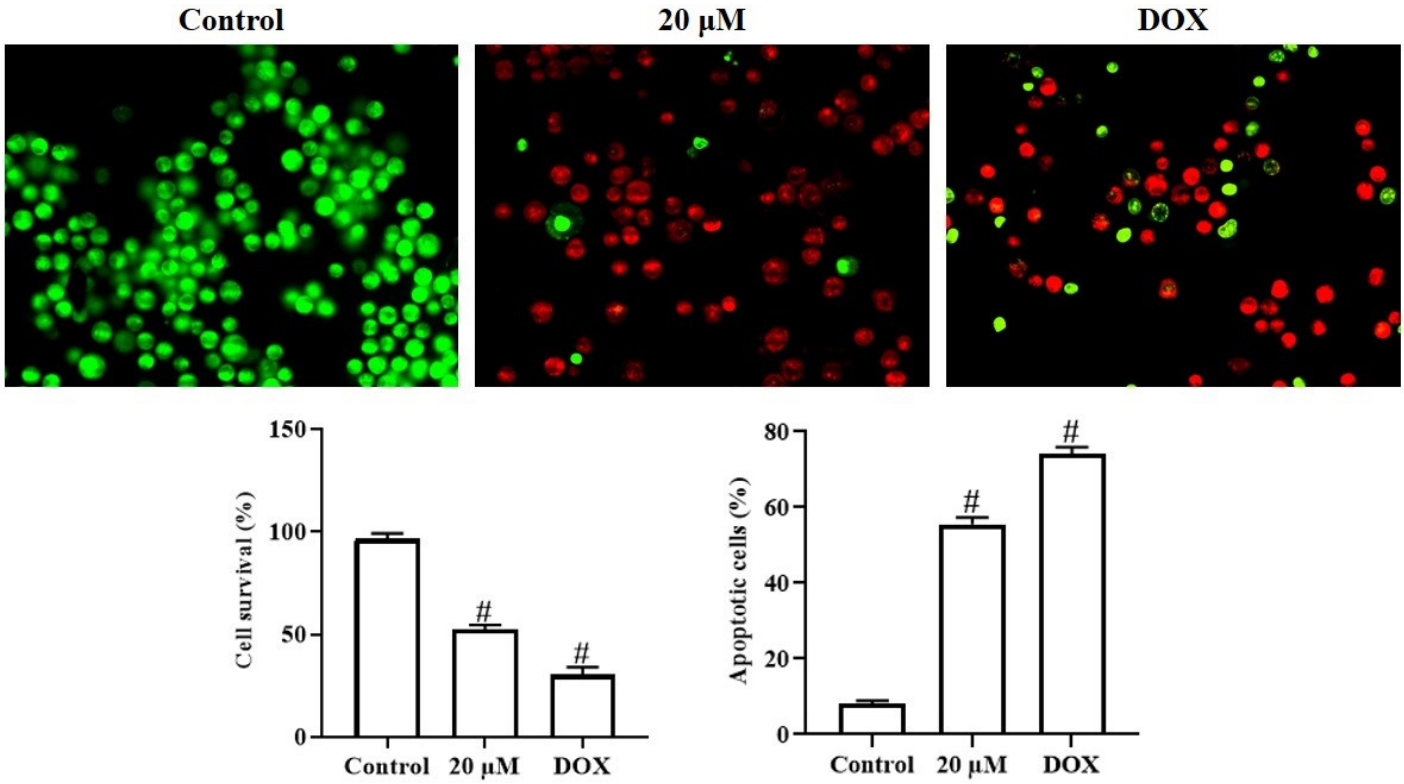

Figure 3: Effect of didymin treatment on the apoptosis in TPC-1 cells. The control cells showed more green fluoresced cells, which reveals more live cells, whereas the didymin-treated TPC-1 cells demonstrated heightened red fluoresced cells, which evidences the onset of apoptosis.

functioning of multicellular organisms, and disruptions in this balance can result in several pathological conditions, including cancer.[23] Apoptosis is defined by a distinct set of morphological and biochemical alterations, which are coordinated by a complex network of signaling cascades involving caspases that act as the executioners of apoptosis. Initiation caspases are triggered via several pathways when cells receive apoptosis signals. Caspases are stimulated, and then associated substrates are degraded, finally resulting in cell apoptosis.[24] The apoptotic process can be initiated by two primary pathways: the extrinsic and intrinsic pathways. The extrinsic cascade is initiated by the stimulation of death receptors, including Fas, TNF-R1, and TRAIL receptors, by their respective ligands.[25] This binding results in the development of a death-inducing signaling complex, which triggers initiator caspases and further activates downstream effector caspases, leading to cell death. The intrinsic cascade, also called as mitochondrial pathway, is triggered in response to intracellular stress signals, including DNA injury and oxidative stress.[26] These stress signals disrupt the mitochondrial membrane potential, leading to the discharge of pro-apoptotic proteins from the intermembrane space, like cytochrome c, which triggers caspase-9 and subsequently activates effector caspases. The

balance between pro- and anti-apoptotic proteins, particularly those belonging to the BCL-2 family, determines the cell's vulnerability to apoptosis.[27]

Cancer cells often evade apoptosis by several mechanisms, including the elevation of anti-apoptotic genes, the decrease of pro-apoptotic genes, and the inactivation of caspases. Deficiencies in the apoptotic machinery can enhance cancer progression by allowing damaged or abnormal cells to survive and proliferate unchecked.[28] Mitochondria play a central role in the intrinsic pathway of apoptosis, acting as a checkpoint for the release of caspase cofactors that commit the cell to apoptosis. Targeting mitochondrial metabolism or stimulating outer mitochondrial membrane permeabilization can induce cancer cell death. Dysfunction in the apoptotic pathways has been extensively documented in cancer, underscoring its critical role in tumorigenesis and treatment resistance.[29] Strategies to induce apoptosis in cancer cells have emerged as promising therapeutic approaches. Many standard cancer treatments exert their cytotoxic effects by triggering apoptosis in tumor cells. Nonetheless, tumor cells can develop resistance to these therapies by further disrupting apoptotic pathways. Therefore, researchers are actively exploring novel approaches to selectively trigger apoptosis in tumor cells while sparing non-malignant cells.[30] In the current study, the dual staining assay results indicated that didymin-induced TPC-1 cells demonstrated increased red fluorescence, which indicates the onset of apoptosis. These findings evidenced the apoptotic activity of didymin on thyroid cancer cells.

Caspases, a family of cysteine-aspartic proteases, play an imperative role in the implementation of apoptosis, which is pivotal for sustaining tissue homeostasis and removing unwanted cells. Apoptosis is a crucial regulatory mechanism in multicellular organisms, relying on caspases. Within the caspase family, initiator caspases, like caspase-9, and effector caspases, like caspase-3, orchestrate the apoptotic process through a cascade of proteolytic events. The apoptotic pathway, which evolves over a longer period, necessitates energy, is closely linked to caspase proteases, and exhibits a less pronounced association with inflammatory responses.[31] Dysfunction of apoptosis is a hallmark of tumors, where malignant cells escape programmed cell death, contributing to uncontrolled growth, tumor development, and resistance to therapy. The balance between pro- and anti-apoptotic signals controls a cell's fate, and cancer cells often exhibit an imbalance that favors survival, making the apoptotic pathway an attractive

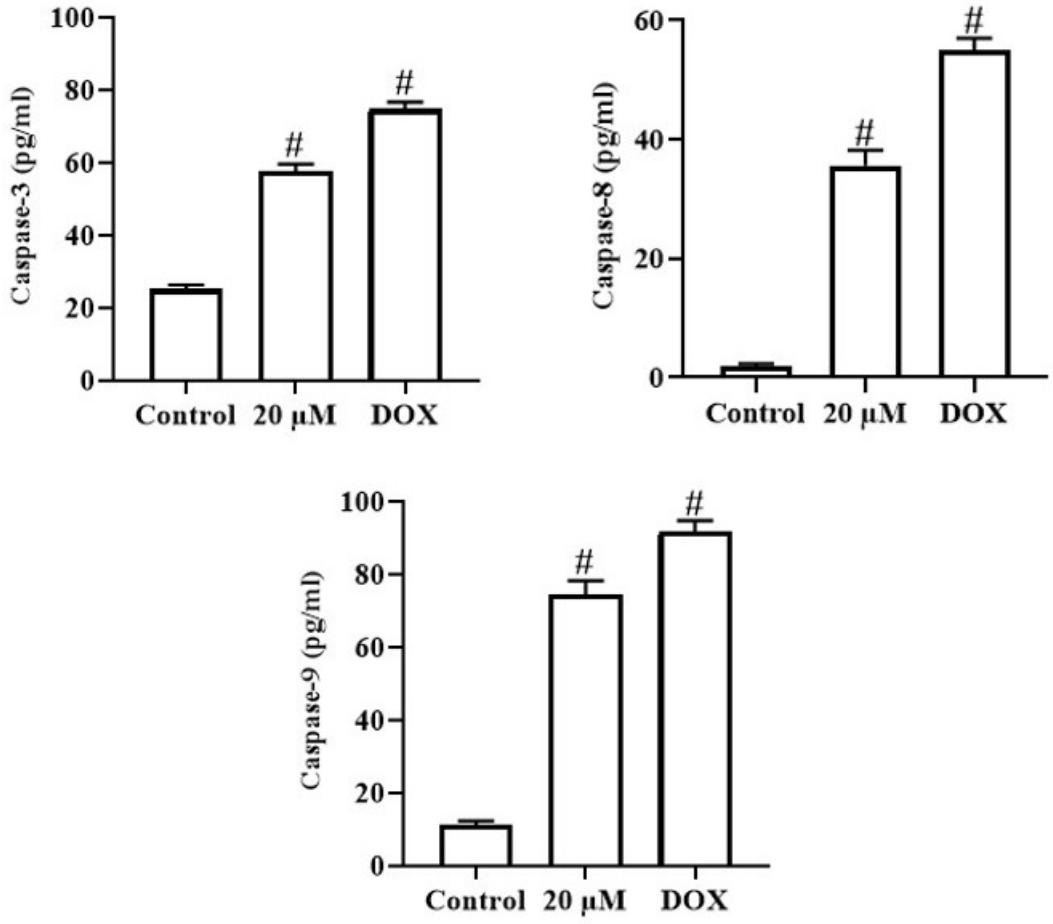

Figure 4: Effect of didymin on the caspase activities in the TPC-1 cells. Values are presented as Mean±SD from triplicate experiments. The results were statistically analysed using one-way ANOVA and Tukey's *post hoc* tests. '#' indicates that values significantly vary from the control group at $p<0.05$.

target for cancer therapeutics. In cancer, suppression of apoptotic pathways arises from elevated levels of anti-apoptotic genes or diminished expression of pro-apoptotic genes, potentially leading to resistance against common anticancer treatments.[32]

Caspases are formed as inactive zymogens (procaspases) that need proteolytic cleavage to become activated, and upon activation, caspases cleave a specific set of cellular substrates, destroying the cells. The stimulation of caspases is closely regulated by intrinsic and extrinsic cascades, both converging on the activation of executioner caspases, primarily caspase-3, which is essential to execute apoptosis.[33] The extrinsic cascade is activated by extracellular signals via death receptors, while the intrinsic pathway largely depends on the mitochondrion. The intrinsic cascade is initiated by intracellular stresses, which trigger the permeabilization of the mitochondrial outer membrane and the discharge of pro-apoptotic markers into the cytoplasm.[34] Cytochrome c release leads to the development of the apoptosome, which leads to the activation of caspase-9, which in turn activates downstream effector caspases, like caspase-3. The permeabilization of the mitochondrial outer membrane is an imperative event in the intrinsic pathway, mediated by the Bcl-2 family of proteins.[35] The contribution of caspases in apoptosis is essential to cancer therapeutics, as stimulating cell death in tumor cells is a major goal of various treatment strategies. By targeting caspases, therapeutic interventions can selectively induce apoptosis in cancer cells, leading to tumor regression and improved patient outcomes.[36] The present findings proved that the treatment with didymin effectively increased the caspase-3/8/9 activities in the TPC-1 cells. These findings highlight that didymin can induce caspase-mediated apoptotic cell death in thyroid cancer cells.

CONCLUSION

In conclusion, our present research demonstrates didymin as a promising antitumor agent for thyroid cancer *in vitro* by reducing cell growth and promoting caspase-mediated apoptosis in TPC-1 cells. The antitumor function of didymin may be due to the initiation of caspase-mediated apoptosis in TPC-1 cells. These findings highlight that didymin can be a promising option for thyroid cancer therapy. Further work is essential to clarify the precise mechanisms that support didymin as a potential antitumor agent for thyroid cancer.

ABBREVIATIONS

DMEM: Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl Sulfoxide; **LDH:** Lactate Dehydrogenase; **AO:** Acridine orange; **EB:** Ethidium bromide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

SUMMARY

Thyroid cancer, a tumor originating from the thyroid gland, impacts a considerable portion of the population, with incidence rates exhibiting an increasing trend globally. The increasing prevalence of thyroid cancer, particularly among women globally, emphasizes the necessity of comprehending this disease. The current research indicates didymin as a viable anticancer treatment for thyroid cancer *in vitro*, since it inhibits cell proliferation and induces caspase-mediated death in TPC-1 cells. These data indicate that didymin may serve as a good candidate for thyroid cancer treatment.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263.
2. Kitahara CM, Schneider AB. Epidemiology of Thyroid Cancer. Cancer Epidemiol Biomarkers Prev. 2022;31:1284-1297.
3. Graceffa G, Salamone G, Contino S, Saputo F, Corigliano A, Melfa G, *et al.* Risk Factors for Anaplastic Thyroid Carcinoma: A Case Series From a Tertiary Referral Center for Thyroid Surgery and Literature Analysis. Front Oncol. 2022;12:948033.
4. Cleere EF, Prunty S, O'Neill JP. Anaplastic thyroid cancer: Improved understanding of what remains a deadly disease. Surgeon. 2024;22:e48-e53.
5. Alhejaily AG, Alhuzim O, Alwelaie Y. Anaplastic thyroid cancer: Pathogenesis, prognostic factors and genetic landscape (Review) Mol Clin Oncol. 2023;19:99.
6. De Leo S, Trevisan M, Fugazzola L. Recent advances in the management of anaplastic thyroid cancer. Thyroid Res. 2020;13:17.
7. Kasemsiri P, Chaisakgreenon P, Vatanasapt P, Laohasiriwong S, Teeramatwanich W, Thongrong C, *et al.* Survival Benefit of Intervention Treatment in Advanced Anaplastic Thyroid Cancer. Int J Surg Oncol. 2021; 2021:5545127.
8. Lorusso L, Pieruzzi L, Biagini A, Sabini E, Valerio L, Giani C, *et al.* Lenvatinib and other tyrosine kinase inhibitors for the treatment of radioiodine refractory, advanced, and progressive thyroid cancer. Onco Targets Ther. 2016;9:6467-6477.
9. Jannin A, Escande A, Al Ghuzlan A, Blanchard P, Hartl D, Chevalier B, *et al.* Anaplastic Thyroid Carcinoma: An Update. Cancers. 2022;14(4):1061.
10. Gibson WJ, Ruan DT, Paulson VA, Barletta JA, Hanna GJ, Kraft S, *et al.* Genomic Heterogeneity and Exceptional Response to Dual Pathway Inhibition in Anaplastic Thyroid Cancer. Clin Cancer Res. 2017;23(9):2367-2373.
11. Yang JW, Zou Y, Chen J, Cui C, Song J, Yang MM, *et al.* Didymin alleviates metabolic dysfunction-associated fatty liver disease (MAFLD) via the stimulation of Sirt1-mediated lipophagy and mitochondrial biogenesis. J Transl Med. 2023;21(1):921.
12. Zhang Y, RuXian G. Didymin, a natural flavonoid, relieves the progression of myocardial infarction via inhibiting the NLR family pyrin domain containing 3 inflammasome. Pharm Biol. 2022;60(1):2319-2327.
13. Ali MY, Zaib S, Rahman MM, Jannat S, Iqbal J, Park SK, *et al.* Didymin, a dietary citrus flavonoid exhibits anti-diabetic complications and promotes glucose uptake through the activation of PI3K/Akt signaling pathway in insulin-resistant HepG2 cells. Chem Biol Interact. 2019;305:180-194.
14. Morelli S, Piscioneri A, Salerno S, Al-Fageeh MB, Drioli E, De Bartolo L. Neuroprotective effect of didymin on hydrogen peroxide-induced injury in the neuronal membrane system. Cells Tissues Organs. 2014;199:184-200.
15. Hung JY, Hsu YL, Ko YC, Tsai YM, Yang CJ, Huang MS, *et al.* Didymin, a dietary flavonoid glycoside from citrus fruits, induces Fas-mediated apoptotic pathway in human non-small-cell lung cancer cells *in vitro* and *in vivo*. Lung Cancer. 2010;68(3):366-74.
16. Singhal J, Nagaprashantha LD, Vatsyayan R, Ashutosh, Awasthi S, Singhal SS. Didymin induces apoptosis by inhibiting N-Myc and upregulating RKIP in neuroblastoma. Cancer Prev Res. 2012;5(3):473-83.
17. Wei J, Huang Q, Bai F, Lin J, Nie J, Lu S, *et al.* Didymin induces apoptosis through mitochondrial dysfunction and up-regulation of RKIP in human hepatoma cells. Chem Biol Interact. 2017;261:118-126.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Method. 1983;65(1-2):55-63.
19. Liu K, Liu PC, Liu R, Wu X. Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Med Sci Monit Basic Res. 2015;21:15-20.
20. Tuttle RM, Haugen B, Perrier ND. Updated American Joint Committee on Cancer/ Tumor-Node-Metastasis Staging System for Differentiated and Anaplastic Thyroid Cancer (Eighth Edition): What Changed and Why? Thyroid. 2017; 27(6): 751-756.
21. Seib CD, Sosa JA. Evolving Understanding of the Epidemiology of Thyroid Cancer. Endocrinol Metab Clin North Am. 2019;48(1):23-35.

22. Filetti S, Durante C, Hartdl D, Leboulleux S, Locati LD, Newbold K, *et al.* Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2019; 1; 30(12):1856-1883.
23. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol. 2019;20(3):175-93.
24. Campbell KJ, Tait SWG. Targeting BCL-2 regulated apoptosis in cancer. Open Biol. 2018;8(5):180002.
25. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol. 2020;17(7):395-417.
26. Valentin R, Grabow S, Davids MS. The rise of apoptosis: targeting apoptosis in hematologic malignancies. Blood. 2018;132(12):1248-64.
27. Bessou M, Lopez J, Gadet R, Deygas M, Popgeorgiev N, Poncet D, *et al.* The apoptosis inhibitor Bcl-xL controls breast cancer cell migration through mitochondria-dependent reactive oxygen species production. Oncogene. 2020;39(15):3056-3074.
28. Chaabane W, User SD, El-Gazzah M, Jaksik R, Sajjadi E, Rzeszowska-Wolny J, *et al.* Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer. Arch Immunol Ther Exp. 2013;61(1):43-58.
29. Opydo-Chanek M, Cichoń I, Rak A, Kołaczkowska E, Mazur L. The pan-BCL-2 inhibitor obatoclax promotes differentiation and apoptosis of acute myeloid leukemia cells. Invest N Drugs. 2020;38(6):1664-76.
30. Sun YL, Jiang WQ, Luo QY, Yang DJ, Cai YC, Huang HQ, *et al.* A novel Bcl-2 inhibitor, BM-1197, induces apoptosis in malignant lymphoma cells through the endogenous apoptotic pathway. BMC Cancer. 2019;20(1):1.
31. Sahoo G, Samal D, Khandayataray P, Murthy MK. A Review on Caspases: Key Regulators of Biological Activities and Apoptosis. Mol Neurobiol. 2023;60:5805-5837.
32. Van Opdenbosch N, Lamkanfi M. Caspases in Cell Death, Inflammation, and Disease. Immunity. 2019;50:1352-1364.
33. Boice A, Bouchier-Hayes L. Targeting apoptotic caspases in cancer. Biochim Biophys Acta Mol Cell Res. 2020; 1867:118688.
34. Von Haefen C, Wieder T, Essmann F, Schulze-Osthoff K, Dorken B, Daniel PT. Paclitaxel-induced apoptosis in BJAB cells proceeds via a death receptor-independent, caspases-3/-8-driven mitochondrial amplification loop. Oncogene. 2003;22:2236-2247.
35. Peng Z, Gillissen B, Richter A, Sinnberg T, Schlaak MS, Eberle J. Enhanced Apoptosis and Loss of Cell Viability in Melanoma Cells by Combined Inhibition of ERK and Mcl-1 Is Related to Loss of Mitochondrial Membrane Potential, Caspase Activation and Upregulation of Proapoptotic Bcl-2 Proteins. Int J Mol Sci. 2023;24:4961.
36. Szmurło A, Dopytalska K, Szczerba M, Szymańska E, Petniak A, Kocki M, *et al.* The Role of Caspases in Melanoma Pathogenesis. Curr Issues Mol Biol. 2024;46(9):9480-9492.

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