Evaluation of *Chelidonium Majus* L. Alkaloid Effect on *VEGF* Gene Expression and Cell Apoptosis in the Burkitt Lymphoma Cell Line

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Abstract

Background: Burkitt lymphoma (BL) is a type of mature B-cell non-Hodgkin's lymphoma that commonly develops in children and young adults. Vascular endothelial growth factor (*VEGF*) is acknowledged as a vital regulator of angiogenesis in both normal and disease states. In light of the adverse effects linked to chemical treatments, this study aimed to explore the anticancer effects of *Chelidonium majus* L. alkaloid on the Daudi BL cell line and to evaluate the expression of the *VEGF* gene.

Materials and Methods: This project was an experimental study. The cytotoxic effects of the alkaloid derived from *Chelidonium majus* L. on the Daudi and normal cells were evaluated using MTT assays (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The induction of apoptosis in cells was measured utilizing Annexin V/propidium iodide (PI) flow cytometry. Additionally, the expression levels of the VEGF gene were determined via a Real-time PCR assay. Data were entered into SPSS, version 21. Student's t-test and ANOVA test were used for comparisons of groups.

Results: The 50% cytotoxic concentration (CC₅₀) of the alkaloid from *Chelidonium majus* L. was found to be 56.35µg/mL in the Daudi cell. Findings from flow cytometry aligned with those from MTT assays. Real-time PCR assay results showed a significant decrease in the *VEGF* gene expression (P<0.009). Effects observed after 48 hours of treatment across various concentrations of *Chelidonium majus* L. alkaloid demonstrated dose-dependency. However, in peripheral blood mononuclear cells (PBMCs) as a control, the alkaloid did not significantly affect the expression of the *VEGF* gene (P>0.05).

Conclusion: The alkaloid is derived from *Chelidonium majusL*. Appears to influence angiogenesis by down-regulating the *VEGF* gene expression, suggesting its potential as a complementary agent in chemotherapy for Burkitt lymphoma. However; further research is required to evaluate the effectiveness of the *Chelidonium majusL*. Extract as a definitive treatment for Burkitt lymphoma.

Keywords: Burkitt lymphoma, Chelidonium majus, Vascular endothelial growth factor

Introduction

Burkitt lymphoma (BL) is classified as a B-cell non-Hodgkin's lymphoma characterized by a high mitotic rate (1). The disease is associated with several factors, including the Epstein-Barr virus (EBV), human immunodeficiency virus (HIV), and chromosomal translocations that result in the overexpression of the c-Myc oncogene (2, 3). The WHO (World Health Organization) categorizes BL into three clinical types: endemic (linked to malaria and EBV), sporadic, and immunodeficiency-associated. Generally, BL can affect the lymph nodes, brain, spinal cord, intestines, kidneys, and other organs and cause tumors in the jaw and the mouth. The significance of angiogenesis in the growth and survival of lymphoma, as well as its

impact on patients with leukemia and other hematologic malignancies, has been recognized since 1994 (4). Recent studies have demonstrated that the treatment of malignant lymphoma with anti-vascular endothelial growth factor (VEGF) agents can enhance cancer cell apoptosis and a reduction in blood vessels (5-9). Without timely treatment, BL can be fatal in children and elderly patients. Common treatments, which have many side effects, include radiotherapy, chemotherapy, and bone marrow transplant (10). While chemotherapy is widely regarded as an adjuvant treatment method for human cancer following surgery, because surgery alone cannot fully replace it, the response rates to these treatments are often low, and the degree of clinical improvement can be minimal. Moreover, severe toxicity and drug resistance are frequent challenges. Given these hurdles, the development of herbal drugs is crucial for advancing the treatment of BL (11-13). In many developed and developing countries, it is common to use medicinal plants to enhance the effectiveness of compounds used in treating diseases. These natural remedies play a significant role in the treatment cancers of and other malignancies (14). Chelidonium majus L. (C. majus), a member of the poppy family (Papaveraceae), is indigenous to Asia and Europe. This plant contains large amounts of isoquinoline and alkaloid secondary metabolites, including sanguinarine, chelidonine, chelerythrine, berberine, and coptisine, with antibacterial, antiviral, and anti-inflammatory properties. Generally, Chelidonium majusL. Is a rich source of compounds with antimicrobial, antitumor, and anti-inflammatory properties (15, 16). Additionally, the alkaloid-rich extract of С. majus eliminates chemotherapyinduced drug resistance and prevents cancer cell proliferation (17). In this study, the aim was to investigate the antiangiogenesis effects of C. majus alkaloid

and the gene expression of the *VEGF* in the Daudi BL cell line.

Materials and Methods

This project was an experimental study. It was determined that the study complied with the ethical principles as well as the national norms and standards for conducting medical research in Iran. IR.IAU.TNB.REC.1402.096

Cell culture:

Daudi cells, В derived from the lymphoblasts of a BL patient, were procured from the Pasteur Institute of Iran. These cells were cultured and maintained in RPMI-1640 culture medium (Gibco, USA), enriched with L-glutamine and 10% fetal bovine serum (FBS, Bio Idea Co., Iran). The culture was maintained under conditions of 5% CO2 and a temperature of 37°C. Peripheral blood mononuclear cells (PBMCs), used as a control, were isolated from a healthy individual using Ficoll-Lymphodex (Inno-Train, Germany). These cells were subsequently cultured in RPMI-1640 medium supplemented with 15% FBS under conditions of 37°C and 5% CO₂(15, 16).

Preparation of C. majus L. alkaloid

The aerial parts of C. majus L. utilized in this study were gathered from the Haraz Amol Forest in Iran. The plant was identified Department at the of Pharmacognosy, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University (Tehran, Iran). Additionally, it was registered with the herbarium under number 1630-AUPF. the For the preparation of C. majus, the collected samples were dried under controlled temperature conditions and subsequently processed into a fine powder. This dry powder was then extracted three times with 80% methanol, which aided in the extraction of active secondary metabolites, such as alkaloids. Following this, 1N HCL and 1N NaCl were added to the methanolic extract and mixed at a temperature of

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25°C. Finally, the alkaline solution obtained from the previous step underwent a chloroform extraction(18, 19). The obtained product was an alkaloid extract.

Cellular cytotoxicity test

The cytotoxic effects of the alkaloid extracted from C. majus were evaluated in both the Daudi cells and PBMCs using the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) assay. The cytotoxic effects of C. majus alkaloid on Daudi and PBMC cells were analyzed through the MTT assay. In the MTT test, the cells were first examined morphologically and seeded at 30,000 cells per well in 96-well plates. Following a 24-hour incubation period, the cells were treated with various concentrations of C. majus alkaloid (10, 20, 40, 60, 80, 100, 120, and 140 μ g/mL) in 200 μ L of RPMI-1640, supplemented with 2% FBS serum in triplicate. After 48 hours of incubation, the cells were centrifuged, and the culture medium was removed. Each well was subsequently filled with 80 µL of RPMI-1640 and 20 µL of MTT solution (0.005 g/mL of PBS). After four hours of incubation that allowed for formazan crystal formation, 100 µL of dimethyl sulfoxide (DMSO) was added to each well. Three wells were designated as blanks, containing only 100 µL of DMSO, while three additional wells containing 100 µL of RPMI without the C. majus alkaloid were used as controls. After a 20-minute incubation period, the absorbance of each well was measured at a wavelength range of 540 nm and 630 nm. The cytotoxicity of all three compounds on Daudi cancer cells, as well as PBMCs, was determined using the following formula:

 $\label{eq:cytotoxicity} \texttt{Cytotoxicity} ~\% = 1 - \frac{\textit{Mean OD Test} - \textit{Mean OD Blank}}{\textit{Mean OD Control} - \textit{Mean OD Blank}} \times 100$

Flow cytometry

Cellular apoptosis was evaluated using cytometry with flow Annexin V/PI staining. Both Daudi cells and normal cells were treated separately for 48 hours at a concentration of 56.35 µg/mL CC₅₀ (50% cytotoxic concentration) of C. majus L. alkaloid for Daudi cells. Following the incubation period, the cells were analyzed by flow cytometry (BD FACS Calibur; Becton-Dickinson, USA) using an apoptosis detection kit. The evaluation was conducted following the instructions provided with the kit.

RNA extraction and cDNA synthesis:

The Daudi cells were separately treated with various concentrations of C. majus alkaloid (10, 30, 56.35, 80, and 100 µg/mL). Following a 48-hour incubation period, RNA was extracted from the Daudi cells using the ROJE Technologies kit, (Iran). by the kit's protocol. The concentration of the extracted RNA was determined using UV spectrophotometry. The RNA was then reverse transcribed using a cDNA synthesis kit (Yekta Tajhiz Azma, Iran), as per the kit instructions.

Quantitative real-time PCR assay

The expression levels of the VEGF gene and GAPDH, which served as an internal control, were quantitatively measured using real-time RT-PCR. This process was conducted a Light Cycler[®] on 96 instrument (Roche Molecular Systems, Germany), using the SYBR Green PCR Master Mix (Yekta Tajhiz Azma, Iran), following the manufacturer's instructions. The calculation of VEGF gene expression was performed using the $\Delta\Delta$ Ct method. The specific primers for the VEGF gene and GAPDH are listed in Table I.

Statistical analysis

Statistical analyses were conducted to determine the significance level. Student's t-test was employed for comparisons between the groups, while an ANOVA test was used for comparisons involving more

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than two groups. Differences in gene expression were analyzed using GenEx 6 software, and statistical analyses were performed using SPSS Version 21. Graphs were also created with GraphPad Prism 8. A P-value of less than 0.05 was considered to indicate statistical significance.

Results

Cytotoxic effects of *C. majus* L. alkaloid on Daudi and PBMC cells

The CC₅₀ of C. majus alkaloid on Daudi cells and PBMCs was determined using the MTT assay after 48 hours. The results of the MTT assay for various concentrations of C. majus alkaloid on Daudi cells and PBMCs are depicted in Figure 1. The CC_{50} value of *C. majus* alkaloid for the Daudi cell line was found 56.35µg/mL after 48 hours. be to Interestingly, the MTT assay results indicated that this concentration of C. majus alkaloid did not exhibit the same cytotoxic effect on PBMCs (normal cells).

Apoptosis assay

The cell apoptosis effects of *C. majus* alkaloid on the Daudi cell line and PBMCs were examined by flow cytometry. The Daudi and PBMC cells were treated with a CC_{50} concentration of *C. majus* alkaloid. As shown in Figure 2, the alkaloid derived

from *C. majus* was found to induce apoptosis in cancer cells. However, it did not have the same adverse effects on normal cells at the same concentration. The results of the Annexin V/PI cell apoptosis assay revealed that the CC_{50} concentration of *C. majus* alkaloid on the Daudi cell line led to 1.46% early apoptosis and 37.94% late apoptosis. In contrast, the same concentration caused only 4.8% early apoptosis and 2.3% late apoptosis in normal cells.

Gene expression analysis by real-time quantitative PCR

The VEGF gene expression was evaluated by the real-time quantitative PCR assay. The analysis of VEGF gene expression after treatment with different concentrations of *C*. majus alkaloid showed that VEGF genes were dosedependently inhibited by C. majus alkaloid (Figure 3). The results showed that in the Daudi cells, the VEGF gene expression decreased after 48 hours of C. majus alkaloid treatment (Table II). In the PBMCs treated with C. majus alkaloid (at the abovementioned concentrations), the VEGF gene expression did not alter significantly.

Gene	Primer sequence (5′→3′)	PCR Product length (bp)	PCR Protocol (Denaturation, Cycles, Extension)	Reference
VEGF*	F: ATCTGCATGGTGATG R: GGGCAGAATCATCACGAAGT	218	95°C/15 min (95°C/20s-58-65°C /20s-72°C/30s)× 5 (95°C/15s-60°C /20s-72°C/20s)× 40 72°C/5 min	(7)
GAPDH	F: CTCTTGCTACTCTGCTCTG R: GCCTGCCTGGTGATAATC	179	94°C/5 min (94°C/40s-56°C/40s, 72°C/45s) × 30 72°C/5 min	(8)

Table I: Sequences of primers and corresponding data for each gene used for real-time PCR

*Vascular Endothelial Growth Factor

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Concentration	Mean (mRNA Fold Change)	SD^*	p-value
10 μg/mL	0.585213	0.12531	0.0291
30 μg/mL	0.524491	0.036519	0.0020
56.35 μg/mL	0.330672	0.109706	0.0088
80 μg/mL	0.291379	0.085738	0.0048
100 μg/mL	0.199212	0.03269	0.0006

Table Π : Statistical analysis of VEGF gene expression in the Daudi cells at different concentrations of C. majus alkaloid after 48 hours.

* Standard deviation



Figure 1. Comparison of CC_{50} obtained for C. majus alkaloid at 48 hours on the MTT assay. The CC_{50} value of C. majus alkaloid on the Daudi cell line was determined to be 56.35 µg/mL. However, this concentration did not have a significant effect on normal cells. The error bars in the graph represent the standard deviation.

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Figure 2. The flow cytometric diagram of Annexin/PI testing. (a) The PBMC line untreated as the control, (b) the PBMC line treated with 56.35 μ g/mL of *C. majus* alkaloid, (c) the untreated Daudi cell line as the control, and (d) the Daudi cell line treated with 56.35 μ g/mL of *C. majus* alkaloid.



Figure 3. Expression of *VEGF* gene in the Daudi cells and PBMCs after 48 hours of treatment. (a) The *VEGF* gene expression is dose-dependently inhibited by different concentrations of *C. majus* alkaloid (*P=0.0291, **P=0.009, ***P=0.0006). (b) There is a significant decrease in the downregulation of the *VEGF* gene compared to the untreated control. The results are expressed as standard deviation (**P<0.009). (c) The expression of the *VEGF* gene compared to the untreated control. The results are expressed as standard deviation (**P<0.009). (c) The expression of the *VEGF* gene compared to the untreated control. The results are expressed as standard deviation (**P<0.009). (c) The expression of the *VEGF* gene compared to the untreated control is not significant in normal cells (P>0.05). (Using Student's t-test and ANOVA test).

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Discussion

Tumor growth and metastasis are dependent on angiogenesis, which is triggered by chemical signals from tumor cells during the rapid growth phase (20). Considering the importance of angiogenesis in the treatment of lymphoma cancer, the VEGF gene has been validated as a therapeutic target in various human cancers (21). Therefore, the development of new therapeutic strategies based on herbal medicines can improve patient This study investigated the survival. cytotoxicity, apoptosis, and gene expression related to the C. majus alkaloid through MTT assays, flow cytometry, and real-time quantitative PCR. The results that С. indicated majusL. Alkaloid decreased the VEGF gene expression in the Daudi BL cell line compared to PBMCs which were used as normal cell controls. In this context, the concentrations of C. majus alkaloid used to inhibit the proliferation of Daudi cancer cells had no harmful effects on normal lymphocytes. Treatment of the Daudi cell line with different concentrations of C. majus alkaloid revealed that C. majus alkaloid inhibited lymphoma cell proliferation in a dose-dependent manner by acting on cellular apoptosis. In 2023, Tuzimski et al. investigated the cytotoxic effects of C. majus on various cancer cells, especially melanoma cells, such as FaDu, SCC-25, MCF-7, and MDA-MB-231. The root extract exhibited the highest cytotoxic activity against melanoma A375 cells, with an IC₅₀ value of $12.65 \mu g/mL$. It also showed significant cytotoxicity against K-MEL-3 cells, with an IC₅₀ value of 1.93 µg/mL (22). Another study by Aljuraisy et al. showed that the aqueous extract of C. majus possessed cytotoxic effects on cancerous cell lines (pelvic RD and cervical HeLa cell lines). The most significant inhibitory efficacy of the extract was observed after 72 hours of exposure to the highest concentration of C. majus (1000 μ g/mL) (23). In this study, it was shown that the alkaloids derived from the aerial parts of C. majus exhibit significant cytotoxicity against the Daudi cell line, after 48h, with a CC50 value of 56.35µg/mL. Additionally, no toxic effects were observed on normal cells. The differences in the results between this study and previous research could be attributed to several factors, including the primary source of the plant used for extraction, the type of extract used, and variations in cell types. Additionally, in 2018. Herrmann et al. demonstrated that chelidonine, an alkaloid found in C. majus, possesses pro-apoptotic and anti-metastatic properties in head and neck squamous cell carcinoma (HNSCC) cell lines. While mucosal keratinocytes were significantly affected by chelidonine, fibroblasts exhibited much greater resistance (24). In the present study, the whole alkaloid of C. majus, which exhibited dose-dependent effects, has been used. Interestingly, normal cells (PBMCs) demonstrated resistance to the alkaloid. Moreover, a study by Abu-Bakr Hassan et al. in 2016 showed that the ethanolic extract of C. majus had an anti-angiogenic potential against Ehrlich Ascites Carcinoma (EAC) in mice. In their study, the EAC cells were introduced into mice, following which mice with solid tumors were treated with C. majus. The activity of pro-angiogenic mediators was then assessed in the serum of various mouse groups (21). In 2007, a study conducted by Basini et al. demonstrated that sanguinarine, an alkaloid derived from C. majus, can inhibit vessel growth. To investigate this, they examined the effect of 300 nM sanguinarine on the production of VEGF in swine granulosa cells. They found that the VEGF signaling pathway was the primary effector (25). In the present study, using the real-time quantitative PCR, it

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was demonstrated that C. majus has an anti-angiogenic effect on BL cells, but does not affect human cells. Other studies have indicated that alkaloids play a crucial role in tumor growth and invasion. For instance, berberine, one of the alkaloids found in C. majus, has been shown to inhibit angiogenesis in breast cancer and hepatocellular carcinoma (26-28). In another study, M Deljanin et al. found that Chelidonium majus crude extract has timedose-dependent activity and against migration and cytotoxic activity against human tumor cell lines A549, H460, HCT 116, SW480, MDA-MB 231, and MCF-7. However, does not affect peripheral blood mononuclear cells from healthy individuals. Cell cycle analysis was performed using flow cytometry and cell morphology assessment, revealing that the extract induces apoptosis. The inhibitory effect of cancer cells was evaluated using the wound healing assay (29). The results of this research are consistent with those of previous studies, with the only difference being the type of tumor cells. In various studies, researchers have demonstrated that chelidonine, a major alkaloid, exhibits potent cytotoxic effects against several types of cancer cells. These include leukemia, PANC-1 (a type of pancreatic cancer, with an IC₅₀ value of 20.7 μ g/mL), and HT-29 cells (a type of colon cancer, with an IC₅₀ value of 20.6 μ g/mL). Furthermore, chelidonine, which was isolated from the ethanolic extract of C. majus, has been reported to inhibit proliferation and induce apoptosis in the HeLa cells (30-32). In 2024, Zhu M et al. indicated that the extract of Celastrus orbiculatus inhibits the proliferation of human Burkitt lymphoma Raji and Ramos cells and promotes apoptosis by regulating apoptosis-related proteins. To evaluate cell viability, they used the CCK-8 assay. Western blotting was employed to detect alterations in apoptosis-related proteins, while annexin V-PI and JC-1 staining

experiments were conducted to assess apoptosis (33). The findings of the aforementioned studies align with the results of this research. By flow cytometry method, we observed that the alkaloid from C. majus L. induced apoptosis in the Daudi cell line, but had no significant Furthermore, effect on PBMCs. it significantly decreased the level of the VEGF mRNA in the Daudi cell line compared to both PBMCs (a normal cell type) and the untreated Daudi cell line, which served as a control.

Conclusion

Based on the findings, the alkaloid from C. majus L. has the potential to suppress VEGF expression in the Daudi cell line. This suggests that this compound could significantly inhibit angiogenesis. Our results offer a stronger theoretical and experimental basis for upcoming in vivo studies. The data discussed here also bolsters the case for C. majus L. a promising new anti-tumor agent. considered Therefore, it may be а candidate drug for use as an adjuvant in the treatment of BL.

Data Availability

The authors declare that all data obtained during this study are available within the manuscript.

Ethical Considerations

This project was found to be in accordance with the ethical principles and the national norms and standards for conducting medical research in Iran. IR.IAU.TNB.REC.1402.096.

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Authors' Contributions

MP and FA conceived and designed the research. MS Carried out experiments and acquired the data. MP and MS interpreted the data and carried out data analysis and statistical analysis. MS wrote the original draft of the manuscript. MP, FA, MHP, and MS modified the paper. All authors read and approved the final manuscript.

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Conflict of Interest

Authors have no conflict of interest to declare.

References

1. Hashemi A, Shahvazian N, Zarezadeh A, Shakiba M, Atefi A. Frequency of Tumor Lysis Syndrome in Aggressive and Slow Introduction Chemotherapy in Children with ALL. IJPHO 2010;1: 19-23.

2. Rowe M, Fitzsimmons L, Bell AI. Epstein-Barr virus and Burkitt lymphoma. Chin. J. Cancer 2014; 33(12): 609-619.

3. Molyneux EM, Rochford R, Griffin B, Newton R, Jackson G, Menon G, et al. Burkitt's lymphoma. The Lancet 2012;379 (9822):1234-1244.

4. Becker J, Covelo-Fernandez A, von Bonin F, Kube D, Wilting J. Specific tumor-stroma interactions of EBV-positive Burkitt's lymphoma cells in the chick chorioallantoic membrane. Vasc cell 2012; 4:1-12.

5. Alshenawy HA. Prognostic significance of vascular endothelial growth factor, basic fibroblastic growth factor, and microvessel density and their relation to cell proliferation in B-cell non-Hodgkin's

lymphoma. Ann Diagn Pathol 2010;14(5):321-327.

6. Ribatti D, Nico B, Crivellato E, Roccaro A, Vacca A. The history of the angiogenic switch concept. Leukemia 2007; 21(1):44-52.

7. Elebiyo TC, Rotimi D, Evbuomwan IO, Maimako RF, Iyobhebhe M, Ojo OA, et al. Reassessing vascular endothelial growth factor (VEGF) in antiangiogenic cancer therapy. Cancer Treat Commun 2022; 32:100620-100631.

8. Cheragh M, Sadeghizadeh Μ, Pouriayevali MH. Parsania M. Dendrosomal nano-curcumin reduces VEGF gene expression with and increasing cell apoptosis has an inhibitory effect on the Burkitt lymphoma cell line. J Hum Genet 2021; 5(1):1-7.

9. Jiang L, Li N. B-cell non-Hodgkin lymphoma: importance of angiogenesis and antiangiogenic therapy. Angiogenesis 2020; 23(4): 515-529.

10. Koster A, Raemaekers JM. Angiogenesis in malignant lymphoma. CURR OPIN ONCOL 2005;17(6):611-616.

11. Li X, Yang X, Liu Y, Gong N, Yao W, Chen P, et al. Japonicone A suppresses growth of Burkitt lymphoma cells through its effect on NF- κ B. Clin Cancer Res 2013;19(11):2917-2928.

12. Ruan J, Hajjar K, Rafii S, Leonard J. Angiogenesis and antiangiogenic therapy in non-Hodgkin's lymphoma. Ann Oncol 2009; 20(3): 413-424.

13. Yazdanparast MS, Ghilian MM, Sheikhpour E. The management and approach to osteosarcoma in children: A mini-review article. IJPHO 2023;13(3):214-221.

14. Ali M, Wani SUD, Salahuddin M, Manjula S, Mruthunjaya K, Dey T, et al. Recent advance of herbal medicines in cancer-a molecular approach. Heliyon 2023; 9(2):13684-13707.

Iran J Ped Hematol Oncol. 2025, Vol 15, No 1, 347-357

15. Ghorbanli M, Fani P, Sateei A. Seasonal changes of alkaloids and phenolic compounds in Chelidonium Majus L. in two habitats. JPEP 2009; 4(15): 65-74.

16. Dereli FTG, Ilhan M, Belwal T. Novel drug targets with traditional herbal medicines: Scientific and clinical evidence: Springer 2022; 121-131 p.

17. Yeap SK, Abu N, Akthar N, Ho WY, Ky H, Tan SW, et al. Gene expression analysis reveals the concurrent activation of proapoptotic and antioxidantdefensive mechanisms in flavokawain B– treated cervical cancer HeLa cells. Integr cancer ther 2017;16 (3): 373-384.

18. Haji Mohammad Ali R, Parsania M, Amin G. Comparison of antiviral effects of methanol and hexane extracts of chelidonium Majus L. against acyclovir resistant herpes simplex Type 1. J Arak Uni Med Sci 2018; 21(3): 42-52.

19. Eshraghi S, Amin G, Fakhri S. Study of antibacterial and phytochemical properties of 12 herb extracts against pathogenic nocardia strains. Vet Res Biol Prod 2009; 22(1):62-73.

20. Ribatti D, Nico B, Ranieri G, Specchia G, Vacca A. The role of angiogenesis in human non-Hodgkin lymphomas. Neoplasia 2013;15 (3): 231-238.

21. Hassan AA-B, Abdel-Rafei MK, Hasan HF. Evaluation of Radiosensitizing and Anti-Angiogenesis Activity of Chelidonium majus Extract in Ehrlich Ascites Carcinoma Transplanted Mice. Pak J Zool 2016; 48(6): 1665-1674.

22. Tuzimski T, Petruczynik A, Plech T, Kaproń B, Makuch-Kocka A, Szultka-Młyńska M, et al. Determination of selected isoquinoline alkaloids from Chelidonium majus, Mahonia aquifolium and Sanguinaria canadensis extracts by liquid chromatography and their in vitro and in vivo cytotoxic activity against human cancer cells. Int J Mol Sci 2023; 24(7): 6360-6381. 23. Aljuraisy Y, Mahdi NK, Al-Darraji M. Cytotoxic effect of Chelidonium majus on cancer cell lines. AJVS 2012; 5(1): 85-90.

24. Herrmann R, Roller J, Polednik C, Schmidt M. Effect of chelidonine on growth, invasion, angiogenesis and gene expression in head and neck cancer cell lines. Oncol Lett 2018; 16(3):3108-3116.

25. Basini G, Santini SE, Bussolati S, Grasselli F. The plant alkaloid sanguinarine is a potential inhibitor of follicular angiogenesis. J Reprod Dev 2007; 53(3): 573-579.

26. Alasvand M, Assadollahi V, Ambra R, Hedayati E, Kooti W, Peluso I. Antiangiogenic effect of alkaloids. Oxid Med Cell Longev 2019;2019(1): 9475908-9475924.

27. Kim S, Oh S-J, Lee J, Han J, Jeon M, Jung T, et al. Berberine suppresses TPA-induced fibronectin expression through the inhibition of VEGF secretion in breast cancer cells. Cell Physiol Biochem 2013; 32(5):1541-1550.

28. Tsang CM, Cheung KCP, Cheung YC, Man K, Lui VW-Y, Tsao SW, et al. Berberine suppresses Id-1 expression and inhibits the growth and development of lung metastases in hepatocellular carcinoma. Biochim Biophys Acta - Mol Basis Dis 2015;1852(3):541-551.

29. Deljanin M, Nikolic M, Baskic D, Todorovic D, Djurdjevic P, Zaric M, et al. Chelidonium majus crude extract inhibits migration and induces cell cycle arrest and apoptosis in tumor cell lines. J Ethnopharmacol 2016; 190: 362-371.

30. Paul A, Bishayee K, Ghosh S, Mukherjee A, Sikdar S, Chakraborty D, et al. Chelidonine isolated from ethanolic extract of Chelidonium majus promotes apoptosis in HeLa cells through p38-p53 and PI3K/AKT signalling pathways. JCIM 2012;10(9):1025-1038.

31. El-Readi MZ, Eid S, Ashour ML, Tahrani A, Wink M. Modulation of multidrug resistance in cancer cells by

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DOI: 10.18502/ijpho.v15i1.17280

chelidonine and Chelidonium majus alkaloids. Phytomedicine 2013; 20(3-4): 282-294.

32. Wouters A, Lardon F, Gravekamp C, Apers S, Pieters L. In vitro and in vivo investigations on the antitumour activity of Chelidonium majus. Phytomedicine 2015; 22 (14):1279-1287.

33. Zhu M, Chu Z, Dai X, Pan F, Luo Y, Feng X, et al. Effect of celastrus orbiculatus extract on proliferation and apoptosis of human Burkitt lymphoma cells. fphar 2024;15:1361371-1361383.

Iran J Ped Hematol Oncol. 2025, Vol 15, No 1, 347-357