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Induction of Apoptosis through S- Phase in Human Breast Cancer MDA-MB231 Cells by Ethanolic Extract of *Dodonaea Viscose L.* -an Iraqi Medicine Plant

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Abstract

Dodonaea viscose is one of Iraqi medicinal herbs has been used for traditional medicine as anti-inflammatory, anti-bacterial, anti-ulcer, analgesic, anti-pyretic**,** and anti-oxidant. Our study was designated to examine the anti-cancer activity of *Dodonaea viscose* leaves ethanol extract against human breast cancer MDA-MB231 cells for the first time. The evaluation was determined through MTT assay, cells morphology, flow cytometry analysis for apoptosis, cell cycle arrest, and mitochondria membrane potential. Our results showed IC50 = 75µg/ml, inhibited proliferation of MDA-MB231 cells. In addition, the results showed induction of apoptosis through cell arrested at S phase, and reduced mitochondria membrane potential with increased of concentrations. These findings suggested that the *Dodonaea viscose* may be promising candidate for preventing of breast cancer and pave the research to identify the responsible small natural molecule for anti- breast cancer activity for first time and further mechanistic studies to the affective proteins.

Keywords: Iraqi medicinal herbs, *Dodonaea viscose* herb, Breast cancer, Breast cancer incidence in Iraq, Apoptosis.

1. Introduction

Dodonaea viscose is one of family Sapindaceae species; the phytopharmacological of Dodonaea viscose leaves present of phenols, flavonoids, tannins, sterols, bitter principles and saponins [1]. *Dodonaea viscose* has been used in traditional medicine as anti-bacterial [2], anti-microbial, anti-inflammatory [3], and anti-ulcer [4]. Furthermore, *Dodonaea viscose* water, ethanol, and methanol extract may provide a pharmacological evidence of pre-diabetic conditions in rats and mediated by interacting with multiple targets operating in diabetes mellitus [5, 6]. In addition, ethanol extract of dodonaea viscose roots identified two new oleanane-type triterpenoid saponins showed anti-proliferative activity against the A2780 human ovarian cancer cell line [7]. Dodoviscin A. isolated from the aerial parts of Dodonaea viscose showed inhibition of

melanin biosynthesis in mouse B16-F10 melanoma cells and may be give a new promising pigmentation-altering agent for cosmetic and therapeutic applications [8]. Viscosine was isolated from *Dodonaea viscosa*, showed a significant lipoxygenase inhibitory activity [9].

Breast cancer is a common disease among women in the worldwide and increased year to year, the incidence estimated more than 1.68 million women were diagnosed and 522,000 women died from breast cancer in 2012, and the mortality is varying in the world [10, 11]. In Iraq, According to the latest Iraqi Cancer Registry, breast cancer account for approximately one-third of the registered female cancers in Iraq (Iraqi National Cancer Research Center, 2013). the incidence rate of all women breast cancer (all ages) increased from 26.6 per 100,000 in 2000 to 31.5 per 100,000 in 2009, risen year to year, and still effects younger age groups than their counterparts in developed countries [12, 13]. The chemoprevention of breast cancer through tamoxifen and raloxifene and side effects of drugs increased the risk [14]. Clinical trials could exam class of natural drugs based on women body nature [15]; therefore, our target is to discover new natural herbs as anti-breast cancer reduces the risk and identify molecules could play a role in breast cancer treatment or prevention. In our designated study, we screen four Iraqi medicinal herbs ethanol extract against breast cancer MDA-MB231 cells for the first time, which are *Dodonaea viscose L., Hibiscus sabdariffa L., Opuntia ficus L., Medicago sativa L.,* and we identified *Dodonaea viscose* with anti-breast cancer activity for the first time, other herbs were negative to breast cancer. We evaluated anti-breast cancer activity of *dodonaea viscose* through MTT assay, cells morphology, Apoptosis, cell cycle arrest, and mitochondria membrane potential.

2. Materials and Methods

2.1. Plant collection

Dodonaea viscose leaves were collected from a grove in Al-Islah area, Thi-Qar province, Iraq, on August 2013, and then cleaned from the soil and washed with distilled water, dried and kept in budgets until use.

2.2. Preparation of ethanolic extract

The dried leaves of *Dodonaea viscose* were crunched and grinded to get a powder crude; 80g of powder crude was added to a thumble and then put in the Soxhlet Continuous Extraction**.** 300 ml of ethanol 96% was added to the round flask of extractor at condition of 70-80 °C and 12 hours. After that, was evaporated by using ethanol solvent by using rotary vacuum evaporator and left it to dry at room temperature and the resultant of 8.89g of *Dodonaea* ethanol extract and kept it in the refrigerator until use.

2.3. Chemicals and reagents for anti-cancer assay

All the chemicals were purchased from Sigma unless otherwise stated. Annexin V-FITC apoptosis detection kit was purchased from promega.

2.4. Cell culture and treatments

Human breast cancer MDA-MB231 cells were maintained in 10 cm plate contained DMEM supplemented with 10% FBS, 100 units/ml penicillin and 100µg/ml/ml streptomycin at 37°C a humidified atmosphere with 5% CO_2. The cells were treated with various concentrations of *Dodonaea viscose* ethanol extract dissolved in dimethyl sulfoxide DMSO with a final concentration less than

1% DMSO- treated cells were used as a control.

2.5. Cell proliferation assay

The effects of *Dodonaea viscose* ethanol extract on cell viability were evaluated by MTT assay as described previously [16]. Human breast cancer MDA-MB231 cells were growth in 96 well/ plate for 24hours and then treated with various concentrations (0-150 µg/ml) of *Dodonaea viscose* ethanol extract for 48 h. Following treatment, the MTT reagent was added (50 µg/ml) and cells were further incubated at 37 °C for 4 h. Subsequently 150 µL DMSO was added to dissolve farmazan crystals and absorbance was measured at 570 nm in a microplate reader (Thermo Scientific).

2.6. Morphological Changes of MDA-MB231 cells

MDA-MB231 cells were growth in 96 well/ plate for 24 h and then treated with 0, 75, 100 and 125µg/ml of *Dodonaea viscose* ethanol extract for 48 h. Morphological changes were observed by phase contrast microscopy (Olympus 1 × 71, Japan).

2.7. Flow cytometric analysis of apoptosis

To determine apoptosis, MDA-MB231 cells were growth in 6 well/ plate for 24h and then treated with 0, 75, 100 and 125 µg/ml of *Dodonaea viscose* ethanol extract for 48 h. The cells were collected, washed with PBS and then re-suspended in binding buffer containing Annexin V-FITC and PI and incubated in the dark for 15 min at room temperature. Then, samples were analyzed by flow cytometry (Beckman Coulter, Epics XL) for the percentage of apoptotic cells.

2.8. Flow cytometric analysis of mitochondrial membrane potential (MMP)

To determine MMP; MDA-MB231 cells were growth in 6 well/plate for 24h and then stained with Rhodamine 123 (Rho-123) as described previously [17]. Briefly, MDA-MB231 cells were incubated with 0, 75, 100 and 125 µg/ml of *Dodonaea viscose* ethanol extract for 48 h. The cells were harvested, washed with PBS, and then incubated with Rho-123 (5 µg/ml) in the dark for 30 min. After washing, the samples were analyzed for the fluorescence of Rhodamine 123 by flow cytometry.

2.9. Flow cytometric analysis of cell cycle arrest

MDA-MB231 cells were growth in 6 well/ plate for 24 h and then treated with 0, 75, 100, and 125 µg/ml of Dodonaea viscose ethanol extract for 48h. The cells were collected, washed with PBS, and fixed with 70% ethanol at 4°C overnight. After washing twice with PBS, the cells were stained with a solution containing 50 µg/ml PI and 100 µg/ml RNase A for 30 min in the dark, at room temperature. Cell cycle profiles were analyzed by flow cytometry (Beckman Coulter, Epics XL).

2.10. Statistical analysis of data

The results are expressed as Mean ± standard error mean (SEM) and statistically compared with control group or within the groups using one way ANOVA followed by Tukey's Multiple Comparison Test. The level of statistical significance was regarded as $P<0.05$. All the experiments were repeated at least three times.

3. Results and Discussion

Breast cancer incidence still increasing in the worldwide especially in the women more than men and the reasons related to the nutrition, genetic inheritance, and

environmental pollution which are caused a mutation of normal breast tissue cells to tumor cells [18, 19]. Nowadays, the researches are focus on identification new small natural molecules from medicinal herbs with new anti-breast cancer activities to develop new anti-breast drugs in prevent or treat breast cancer cells and reduce the side effects [20-22]. In Iraq, breast cancer is ranking in the top of common cancers and followed by colon and bladder cancers and caused by risk factors nutrition and environmental pollution [23, 24]. Dodonaea viscose herb extract is one of medicinal herbs have been used in the traditional medicine in the worldwide [25] and the investigations of it action in cancer activity still unknown for breast cancer. In our study, we evaluated anti-breast cancer activity of *Dodonaea viscose* ethanol extract against human MDA-MB231 cells for the first time. The cells were treated with varies doses of *Dodonaea viscose* ethanol extract for 48h to estimate IC50 and we identified it to be 75µg/ml as showing in figure 1. In the next evaluation, we used four doses (0, 75, 100, 125) µg/ml.

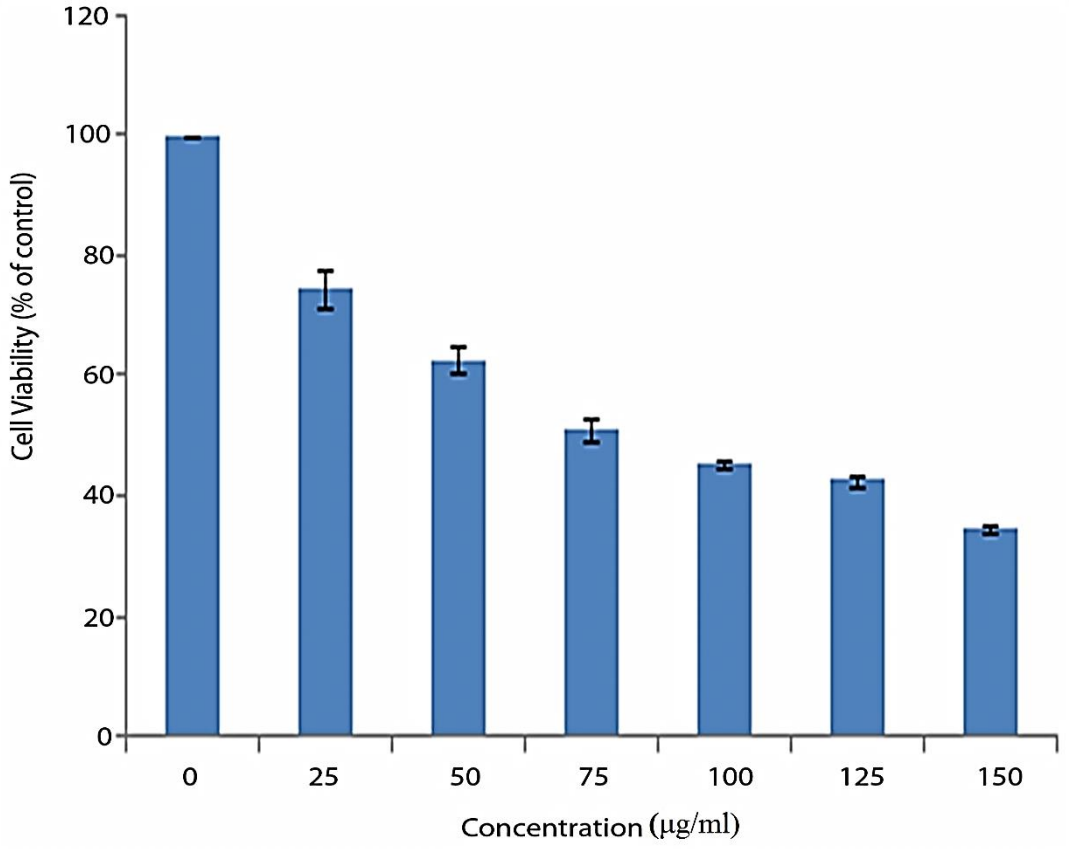

Figure. 1. MDA-MB231 cells were treated with various doses of *Dodonaea viscose* ethanol extract for 48 h, and cells viability was measured by MTT assay. Data are expressed as Mean ± SEM of three independent experiments. Columns not sharing the same superscript letter differ significantly ($P < 0.05$).

To identify whether IC50 has toxicity on MDA-MB231 cells, the cells were treated with four doses above for 48h to observe the morphology changes of MDA-MB231 cells, and we noticed a significantly effect of IC50 with increasing of doses on cells morphology compared with the control, as showing in figure 2.

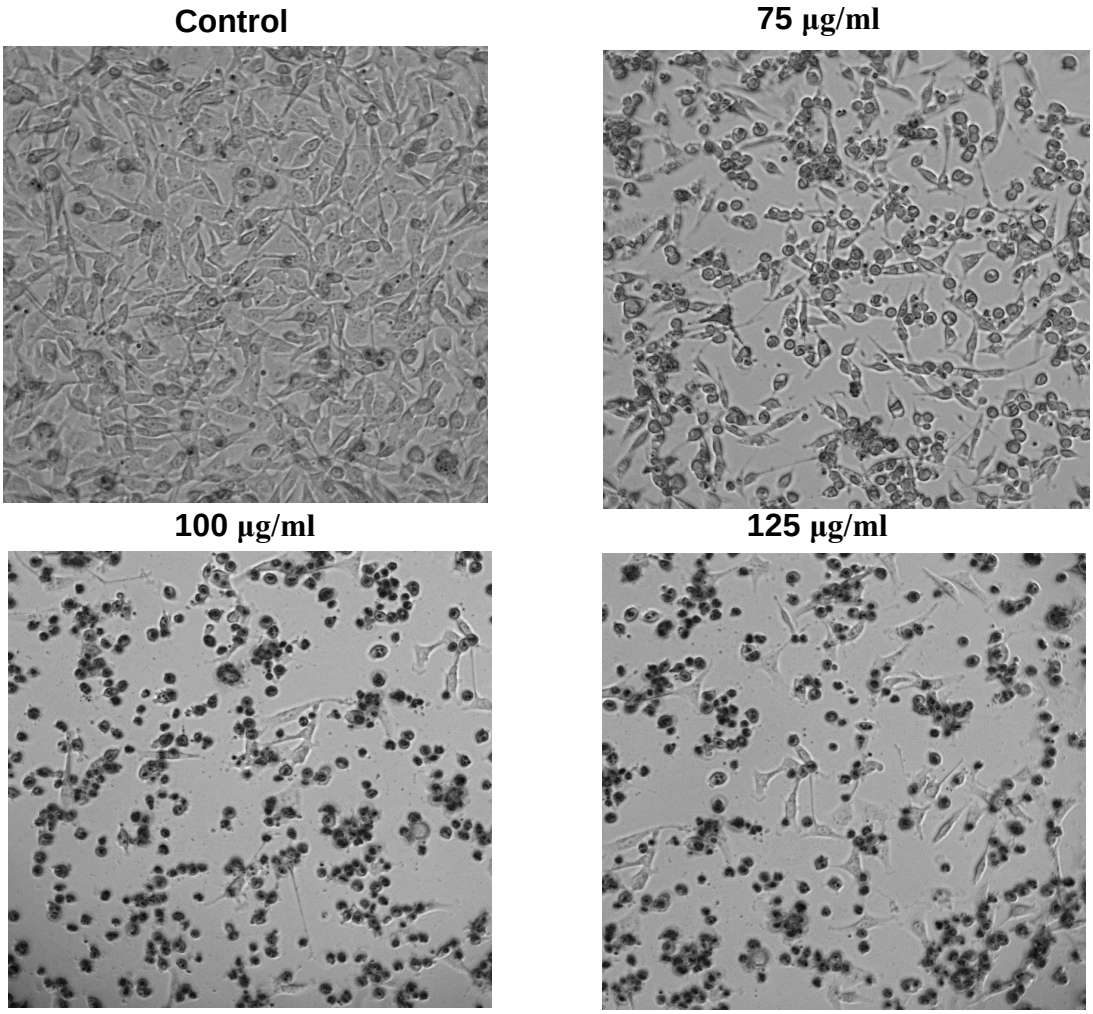

Figure 2. Microscopic analysis of MDA-MB231 cells; the cells were treated with 0, 75, 100 and 125 µg/ml of *Dodonaea viscose* ethanol extract for 48 h, and then observed the morphology changes.

To examine mitochondria membrane potential, the cells were treated with four doses above and analyzed with flow cytometry apparatus. We observed membrane potential decrease with increased doses (0 = 97.05%; 75= 88.43%; 100= 81.014; 125= 72.96%), as showing in figure 3. The released proteins from weak mitochondria membrane potential pave to study the proteins mechanistic in apoptosis action in future.

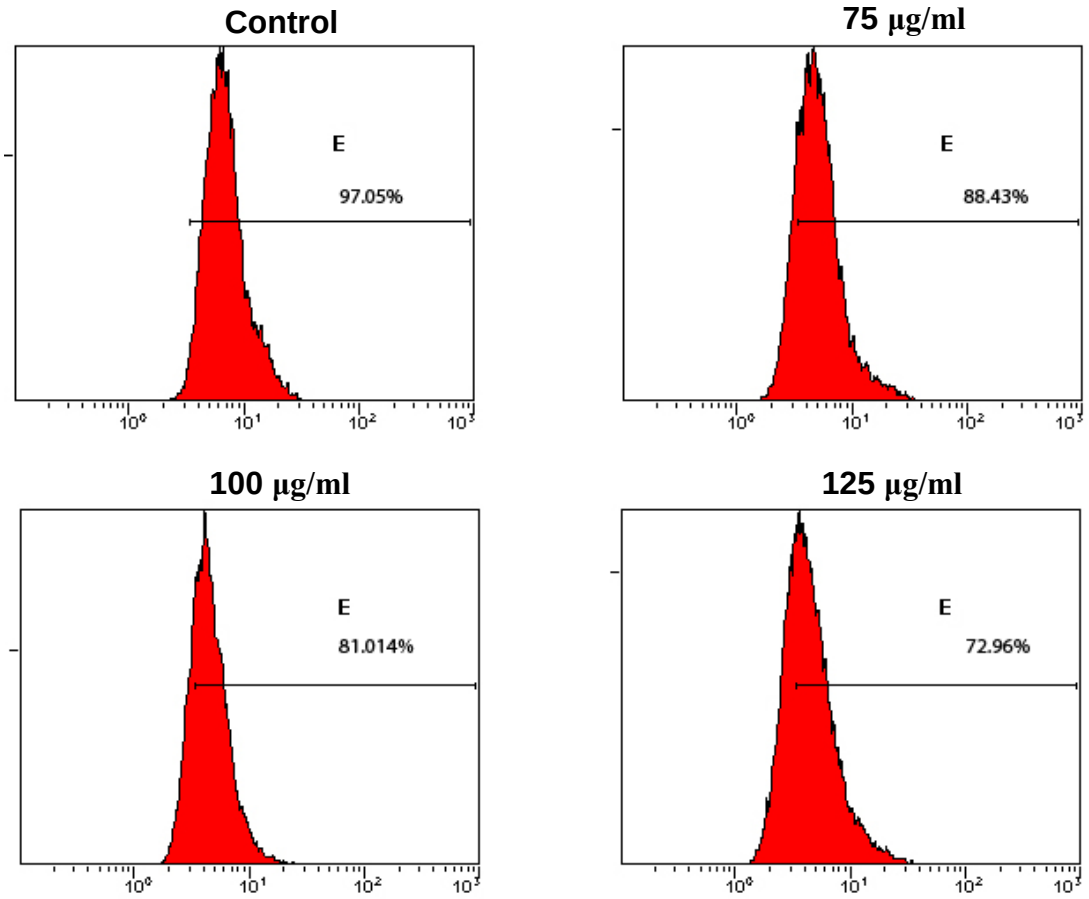

Figure 3. The mitochondrial membrane potential in MDA-MB231 cells, the cells were treated with 0, 75, 100 and 125 µg/ml of dodonaea viscose ethanol extract for 48 h. Data are expressed as Mean ± SEM. of three independent experiments. Columns not sharing the same superscript letter differ significantly (*P* < 0.05).

Therefore; we have done apoptosis analysis via flow cytometry analysis, the cells were treated with four doses above for 48h, and we observed late apoptosis (B2) increased with increased doses, at 75 = 0.85% and 125 = 1.59% compared with the control = 0.75%, in addition, live cells (B3) decreased with doses increasing, as showing in figure 4.

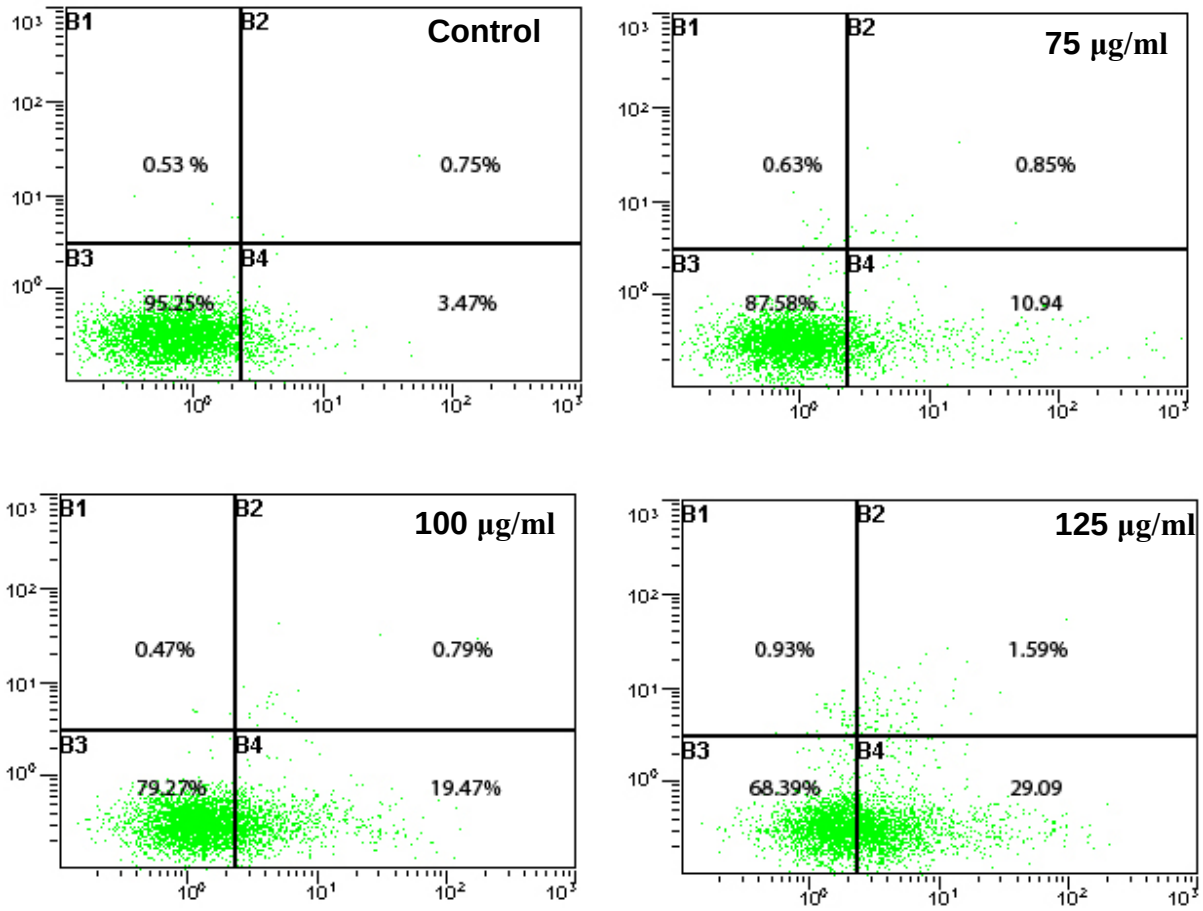

Figure 4. Flow cytometric analysis of apoptosis in MDA-MB231 cells, the cells were treated with 0, 75, 100 and 125 µg/ml of *Dodonaea viscose* ethanol extract for 48 h. Data are expressed as Mean ± SEM. of three independent experiments. Columns not sharing the same superscript letter differ significantly ($P < 0.05$).

To complete the evaluation of *Dodonaea viscose* ethanol extract against human breast MDA-MB231 cells to the last but not least, we have done cell cycle arrest to observe *Dodonaea viscose* ethanol extract arrest cells at which checkpoint G0/G1, or S, or G2/M. The cells were treated with four doses above for 48h, and we analyzed the data by flow cytometry apparatus. The data analysis showed that doses arrest cells at S phase (0 = 33.021%; 75 = 59.766%; 100 = 66.314%; 125 = 70.283%), S phase increased with doses increasing and S phase confidence is good comparing with less values changing of G1, and G2, as showing in figure 5.

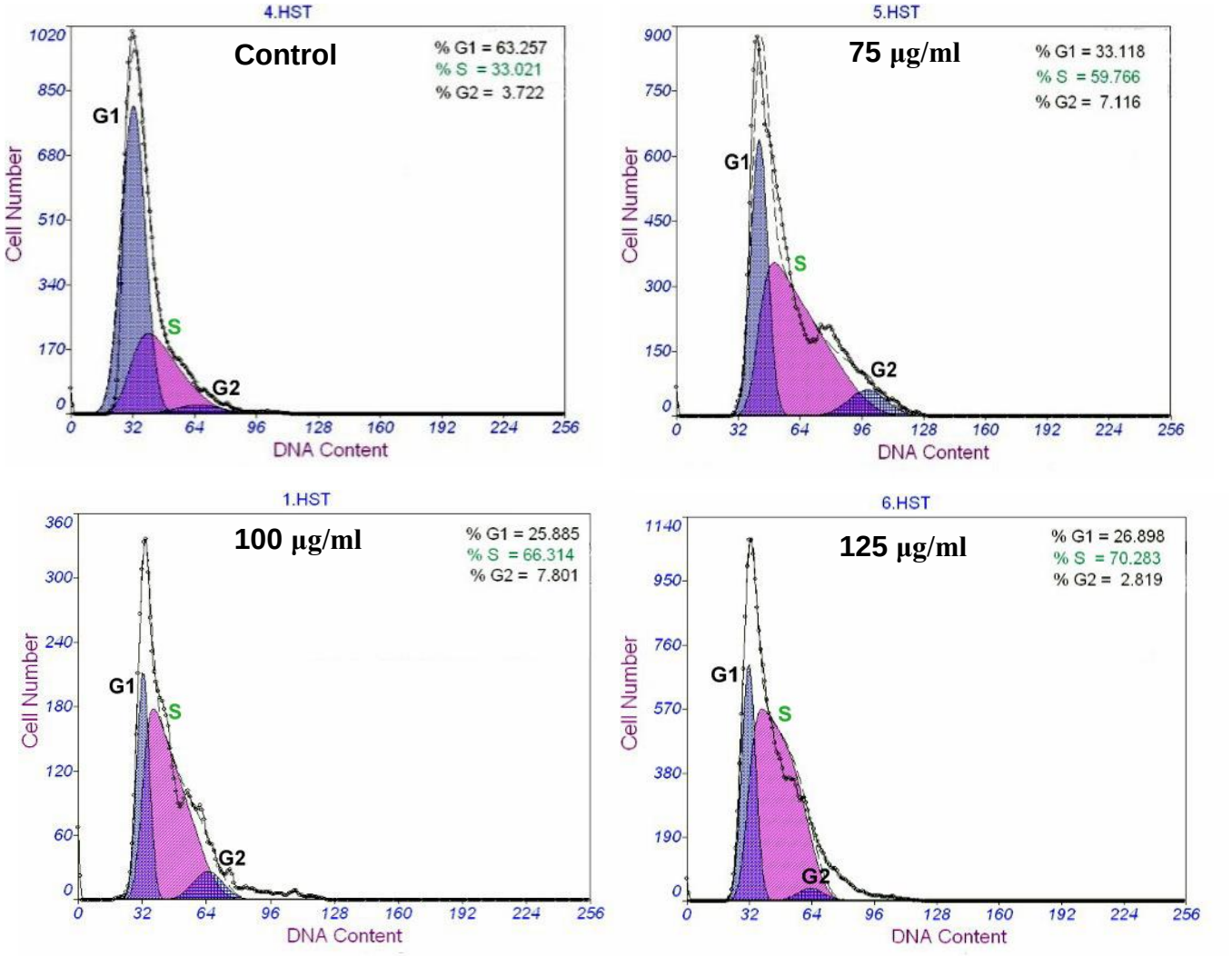

Figure 5. Flow cytometric analysis of the cell cycle distribution in MDA-MB231 cells, the cells were treated with 0, 75, 100 and 125 µg/ml of *Dodonaea viscose* ethanol extract for 48 h. Data are expressed as Mean ± SEM of three independent experiments. Columns not sharing the same superscript letter differ significantly ($P < 0.05$).

In conclusion, the results of *Dodonaea viscose* ethanol extract against human breast MDA-MB231 cancer cells showed a significant effect rising of promising *Dodonaea viscose* herb as a candidate in chemo prevent of breast cancer via nutrition system and could examine it action with against other tumors. Furthermore; future studies focus on identified the responsible small natural molecules from *Dodonaea viscose* extract anti-breast cancer for first time and study it action.

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References

[1] Sandhya, R.; Pippalla, R. and Mohan, K. (2009). Dodonaea Viscosa L. -an Overview**.** JPRHC, Vol. 1, No.1: 97-112.

[2] Mrudula, P.; Roxanne, N. and Foluso, O. (2013). Inhibitory Effect of Dodonaea viscosa var. angustifolia on the Virulence Properties of the Oral Pathogens Streptococcus mutans and Porphyromonas gingivalis. Evidence-Based Complementary and Alternative Medicine, Article ID 624089, 5 pages.

[3] Getie, M.; Gebre, P. and Mariam E. (2003). Evaluation of anti-microbial and anti-inflammatory activities of medicinal plants *Dodonaea viscosa*, Rumex nervosus & Rumex abyssinicus, Fitoterapia Vol 74 (1-2): 139-143.

[4] Veerapur V.; Prabhakar K.; Thippeswamy B.; Bansal B.; Srinivasan K. and Unnikrishnan M. (2010). Anti-diabetic effect of Dodonaea Viscose, aerial parts in high fructose- fed insulin resistant rats: A mechanism based study. Indian journal of experimental biology, Vol. 48: 800-810.

[5] Veerapur, V.; Badiger, A.; Joshi, S.; Nayak, V. and Shastry, C. (2004). Anti-ulcerogenic activity of various extracts of Dodonaea viscosa (L) Jacq. leaves, Indian J. of Pharmaceutical Sciences. Vol.66, 4: 407-411.

[6] Jangra, M.; Sharma, S. and Kumar, M. (2011). Evaluation of Anti-hyperglycemic Activity of Dodonaea Viscosa Leaves in Normal and Stzdiabetic Rats. Int. J. Pharm Sci., Vol 3, 3: 6974.

[7] Shugeng, C.; Peggy, B.; Martin, C.; Richard, R.; Jeremi, R.; Etienne, R. and Vincent, E. (2009). Anti-proliferative Triterpenoid Saponins of Dodonaea viscosa from the Madagascar Dry Forest1. J. Nat Prod. 72(9): 1705–1707.

[8] Yan, G.; Zhu, J.; Zhang, L.; Xu, Z.; Wang, G. and Zhu, W. (2013). Dodoviscin A. inhibits melanogenesis in mouse B16-F10 melanoma cells. Planta Med. 79 (11): 933-8.

[9] Amir, Z.; Akhtar, M. Zafar, I.; Itrat, A.; Muhammad, R. and Said, N. (2013). Molecular docking of viscosine as a new lipoxygenase inhibitor isolated from Dodonaea viscose. Bangladesh J. Pharmacology 8: 36-39.

[10] Omid, B.; Per, H.; Anders, E. and Tahereh, M. (2012). Breast cancer incidence and case fatality among 4.7 million women in relation to social and ethnic background: a population-based cohort study. Beiki et al. Breast Cancer Research 14: 5.

[11] American Cancer Society (2013). Breast Cancer Facts & Figures 2013-2014. Atlanta: American Cancer Society, Inc.

[12] Muzahem, M. and Xiang, J. (2014). Breast cancer in Iraq, Incidence trends from 2000-2009. Asian Pac J. Cancer Prev., 15 (1): 281-286.

[13] Hamid, Y. and Sharif, F. (2014). Incidence Rates, Pattern and Time Trends of Registered Cancer in Iraq (1991-2008) Population and Hospital Based Registry. Open Access Library Journal 1: 1-6.

[14] Nelson, H.; Smith, M.; Griffin, J. and Fu, R. (2013). Use of medications to reduce risk for primary breast cancer: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 158(8): 604-614.

[15] Goss, P.; Ingle, J. and Ales-Martinez, J. (2011). Exemestane for breast-cancer prevention postmenopausal women. N. Engl. J. Med. 364(25): 2381-2391.

[16] Rasul, A.; Khan, M.; Yu, B.; Ma, T. and Yang, H. (2011). Xanthoxyletin, a Coumarin Induces S-Phase Arrest and Apoptosis in Human Gastric Adenocarcinoma SGC-7901 Cells. Asian Pac. J. Cancer Prev. 12: 1219–1223.

[17] Muhammad, K.; Bo, Y.; Rasul, A.; Al Shawi, A.; Yi, F.; Hong, Y. and Ma, T. (2012). Jaceosidin Induces Apoptosis

in U87 Glioblastoma Cells through G2/M Phase Arrest. Evidence-Based Complementary and Alternative Medicine, Article ID 703034, 12 pages.

[18] John, E.; Phipps, A.; Davis, A. and Koo, J. (2005). Migration history, acculturation, and breast cancer risk in Hispanic women. Cancer Epidemiol Biomarkers Prev. 14: 2905-2913.

[19] Zackrisson, S.; Andersson, I.; Manjer, J. and Janzon, L. (2004). Non-attendance in breast cancer screening is associated with unfavourable socio-economic circumstances and advanced carcinoma. Int. J. Cancer Prev.108: 754-760.

[20] Om, P.; Ateeque, A.; Vinay, K.; Sudeep, T.; Aditya, B. and Feroz, K. (2014). In Silico Assay Development for Screening of Tetracyclic Triterpenoids as Anticancer Agents against Human Breast Cancer Cell Line MCF7. PLOS ONE Vol. 9, Issue 11: 111049.

[21] Ruddy, K. and Winer, E. (2013). Male breast cancer: risk factors, biology, diagnosis, treatment, and survivorship. Ann Oncol. 24(6):1434- 1443.

[22] American Cancer Society (2015). Breast Cancer Overview. Atlanta: American Cancer Society, Inc.

[23] Alwan, N. (2010). Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. East Mediterr Health J. 16: 1159-64.

[24] Iraqi Cancer Board (2010). Results of the Iraqi Cancer Registry 2010. Baghdad, Iraqi Cancer Registry Center, Ministry of Health.

[25] Venkatesh, S.; Reddy, Y.; Ramesh, M.; Swamy, M.; Mahadevan, N. and Suresh, B. (2008). Pharmacognostical studies on Dodonaea viscose Leaves. Afr. J. Pharm. Pharmacol. 2: 83-88.

تثبيط الموت الخلوي المبرمج عند الطور S لخلايا سرطان الثدي البشري نوع MDA-MB231 بواسطة المستخلص الايثانولي لأوراق النبات الطبي العراقي نبات الدودنيا

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الخلاصة

يعتبر نبات الدودنيا واحد من النباتات الطبية العراقية المستخدمة في العلاج الطبي كمضاد للالتهابات والبكتريا والقرحة والحمى والاكسدة ومسكن للألم.

صممت الدراسة الحالية لاختبار فعالية المستخلص الايثانولي لأوراق نبات الدودنيا ضد خلايا سرطان الثدي البشري نوع MDA-MB231 لأول مرة.

تقييم الفعالية السرطانية تم من خلال حساب التركيز المثبط الفعال IC50 باستخدام فحص MTT assay والتغير في هيئة ونمو الخلايا ونسب الموت المبرمج والدورة الخلوية والتغير في جهد مقاومة غشاء المايتوكوندريا باستخدام تقنية القياس الخلوي بالجريان للخلايا.

اظهرت النتائج بان التركيز المثبط هو 75مايكرو غرام/مل حيث ثبط نمو الخلايا مع زيادة التركيز، بالإضافة الى زيادة نسب الموت المبرمج للخلايا السرطانية مقارنة بالخلايا الحية من خلال تأثيره على الطور S من اطوار دورة الخلية. كما انخفض جهد مقاومة غشاء المايتوكوندريا مع زيادة التركيز.

ان هذه النتائج اقترحت بان نبات الدودنيا قد يوعدنا في منع سرطان الثدي مستقبلا ويدفعنا في البحث المستقبلي الى عزل وتشخيص المركب الطبيعي المسؤول عن الفعالية السرطانية ضد سرطان الثدي البشري لأول مرة ودراسة الميكانيكية للبروتينات المتأثرة مستقبلا.

الكلمات المفتاحية: النباتات الطبية العراقية، نبات الدودنيا، سرطان الثدي، سرطان الثدي في العراق، الموت الخلوي المبرمج