

***Datura stramoium* Extract Inhibits Mitosis in Cancer Cells**

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Abstract

The aim of this study was to evaluate the anti mitotic activity of ether extract prepared from *Datura stramoium* plant on cancer cells.

Recently oncology studies are directed toward substances which act somehow to induce apoptosis or to prevent abnormal dividing of cell, that may provide therapeutic strategies for the treatment of cancer. Thus, many research on various types of plants such as fruits, vegetables, other edible plants and toxic plants have been done *in vitro* and *in vivo*. But, few studies on *Datura* plant were occurred and there was no previous study dealing with its effect on proliferative activity of cancer cells.

In current study, two types of common malignant tumor were tested *in vitro*. These were mammary cancer and glioma. The cancer cells were exposed to various concentration of ether extract of *Datura stramoium* 1000, 1250, 1500 µg/ml. Then percentage of mitotic indexes were estimated.

The results exhibited that there were decreases in percentages of mitotic index in both types of cancer cells. However, the decrement was significant in the mammary cancer and insignificant in glioma cell line.

Conclusion: Conclusion obtained from this study indicated that ether extract study of *Datura stramoium* has ability to inhibit mitotic activity of cancer cells.

Key words: *Datura stramoium*, Antiproliferative agent, Antimitotic agents.

Introduction

Cell can be divided through entering cell cycle. The cell cycle consists of four distinct phases: G₁ phase, S phase, G₂ phase (cell actively known as interphase, during which cell grows, accumulation nutrients needed for mitosis and duplication its DNA) and M phase in which the cell chromosomes are divided between two daughter cell and the cytoplasm divides forming distinct cell [1].

Any changes or aberration in this regulatory system of cell division may lead to uncontrol cell division giving rise to malignancy defining a cancerous/neoplastic growth [2]. Thus suppression of abnormal cell division may provide therapeutic strategies for the treatment of inappropriate cell proliferation or decrease the rate of recurrence risk or increase rate of patient survival [3]. Therefore the research is directed toward substances which act somehow to induce apoptosis or to prevent cell from division. These substances may be plant poisoning or extracted from microorganism. Such as lunasin is a novel peptide identified in soybean has ability to suppress carcinogenesis of skin and other mammalian cells by chemical carcinogens [4]. Virgin olive phenol extract inhibits proliferation of promyelocytic leukemia cells (HL60) and induce apoptosis [5] whereas phenol and hexan extracts of Mastic gum contain constituents which can induce P₅₃ and P₂₁ independent G₁-phase arrest followed by apoptosis of human HCT 116 colon cancer cells *in vitro* [6]. Studies of *Nigella sativa* and *Crocus sativus* extracts, showed that these plants have ability to inhibit two stages, initiation,

promotion [dimethylbenzena anthracene/croton oil] skin carcinogenesis in mice [7]. While others repeated that six green tea catechins and caffeine including antimutagenesis [8].

D. stramoium is a wild growing flowering plant belongs to the family solanaceae and is a medicinal plant with antinociceptive, antioxidant, hypolipidemic, anti-inflammatory, antirheumatoid and hypoglycemic [9]. On the other hand, many studies on *D. stramoium* showed that it has ability to act *in vitro* and *in vivo* against cancer cells. The aqueous *D. stramoium* leaf extracts showed cytotoxic effect on breast (MDA- MB231), head, neck (FaDu) and lung (A549) cancer cell lines [10]. Whereas acetone and ether extract of *D. stramoium* seeds produced cytotoxicity on laryngeal cancer cell (Hep₂), mammary (AMN3) and glioma cell lines [11,12]. *In vivo*, *D. stramoium* seed extracts exhibited their ability to reduce the size and doubling rate of mammary cancer. In addition, ether extract prevented metastasis. Moreover, the prophylotic study showed that the extracts of *D. stramoium* reduce the percentage of mice bearing tumor in mice treated with these extracts prior to implantation with mammary tumor [13].

The purpose of this study is to evaluate the effect of the ether extract of this plant on cell division of cancer cell lines.

Materials and Methods

Preparation of seeds extract: The extract was prepared from *Datura stramoium* seeds, as described by Gaspori-Compani *et al.* [14]. Briefly, seeds were ground several times with diethyl ether in blender. The resulting powder was extracted over night with 10 volume of cold 0.2M NaCl containing 0.005M- sodium phosphate buffer, pH 7.2. After centrifugation, the supernatant was fractionated with (NH₄)₂SO₄ at 0-40%, 40-60% and 60-100% saturation and the precipitates obtaining were redissolved in phosphate buffered saline and dialysed against the same solution.

Cell lines handling: Two types of cancer cell lines were used in this study. These were mammary cancer (AMN3). It was provided kindly by Iraqi Center for Cancer and Medical Genetic Research and glioma cell lines provided kindly by Dr. A. Al-Shimary/Iraqi Center for Cancer and Medical Genetic Research.

Three concentrations of the ether extract were used 0, 1000, 1250, 1500 µg/ml for treating the cells.

The handling of each cell line for each concentration was occurred according to [15] with slight modification.

-The cell line was cultured in falcon by using RPMI media containing 10% of fetal calfe serum at 37°C for 24 hours (using 2 falcons for each concentration and 2 for control).

- By using growth medium. Five ml at each concentration were prepared from extract.

- The cell line was treated with extract after medium in each falcon was poured off then incubated at 37°C for 72 hrs i.e. when confluent monolayer was formed.

- The medium containing extract was replaced (in each falcon) by growth medium containing 20% fetal calfe serum, then after 6 hours from changing medium, 0.2 ml colchicin was added to 5 ml of medium.

- At the end time, cells were harvested by adding 2-3 ml of trypsin-versene and incubated at 37°C for 2-3 minutes and put it in tube which containing media.

- Centrifugation at 1500 rpm for 10 minutes was done.

- The supernatant at each tube was decanted off by Pasteur pipette and the cells were suspended in 10 ml of 0.075M KCl. Potassium chloride was added on the wall of tube with slowly mixing and tubes incubated in water bath at 37°C for 30 minutes.

- After centrifugation at 1500 rpm for 10 minutes, supernatant were removed and cells washed many times with fixative solution (composed from 3 parts methanol+1 part glacial acetic acid). The fixative prepared at the time of using and should be cooling before using.

- After solution became clear 2-3 drops were dropped on wet chilled and grease free glass slide, and left slides for drying
- Slides were stained using freshly made working Giemsa stain (prepared by adding 1 part of Giemsa stain to 4 parts of Sorenson's buffer) which was applied for 2 minutes then rapidly washed with Sorenson's buffer, thereafter left to dry at room temperature.
- Microscopic examination under high magnification using 40X objective lens was performed to determine mitotic index by counting the number of dividing cells counted with the total number of cells (more than 1000 cells were counted). The mitotic index was calculated as follows[16]:

$$\text{Mitotic Index (\%)} = \frac{\text{number of dividing cells}}{\text{Total counted cells}}$$

Results

In mammary cancer cell line, the result exhibited that the higher percentage of mitotic index appeared in control 1.96±0.26% when there was no extract in the medium (zero concentration). Whereas the percentages became decrement in mitotic index when the cells exposed to different concentration of *Datura* extract.

The statistic analysis showed that there were insignificant decrement at 1000, 1500 µg/ml 1.44±0.62 and 1.90±0.5%, respectively. While significantly decrement at level P<0.01 at concentration 1250µg/ml 0.32±0.08% fig (1).

Fig. (2) illustrated the percentage of mitotic index of glioma cell line. There were insignificantly decrement in the mitotic index when the cells exposed to extract were 1.29±0.25, 1.23±0.15 and 1.5±0.34% at 1000, 1250 and 1500 µg/ml, respectively.

As shown in figure (3), there was significant differences at level P<0.05 in mitotic index between mammary cancer cell line and glioma cell line 0.32±0.08, 1.23±0.15% at concentration 1250 µg/ml.

Discussion

A major objective of this study was to investigate whether the ether extract of *D. stramonium* acts as antiproliferative agent. The results reflected that the ether extract components had ability to reduce mitosis of tested cancer cell lines (mammary cancer and glioma). However, the reduction was appeared significantly in mammary cancer and insignificantly in glioma. These observations indicate that the ether extract was more effective on mammary cancer than glioma.

The current investigation appeared that the ether extract has antitumor activity and exerted its activity by inhibit mitosis. Few studies on this plant had been done as antitumor [11], whereas there were no previous studies on it as antiproliferative agent.

The anticancer effect of *Datura* extract was supported by previous study in which appeared that high concentration of the ether extract exhibited cytotoxic effect on different cancer cell lines, but not on normal cell line [12]. And few other studies showed the same action of other types of extract prepared from *D. stramonium* plant against various types of cancer cell lines [10,12].

Previous study illustrated that the components of ether extract were flavenoids, tanrin, lectin and trace amounts of alkaloids[17]. Flavenoids are group of naturally occurring polyphenole compounds present in fruits, vegetable and other edible plants[18]. Research on phytochemicals showed that some flavenoids process chemopreventive properties and numerous studies were performed recently to assess the mechanisms whereby each flavenoids prevent cancer, including induction of cell cycle arrest, apoptosis and antiproliferation[16,19]. Some flavenoids induce G1 and G2 arrest, by binding to and inhibiting the activity of cycline

dependent kinase (CDK). That leads to decrease phosphorylation of retinoblastoma (Rb)[21]. Other types of flavonoids can decrease E2F-1 level which may contribute to cell cycle arrest at G1, whereas their activity to decrease Cdc2 make cell cycle arrest at G2[21]. Other flavonoids inhibit proliferation via inhibit epidermal growth factor (EGF) induced and constitutively active mitogen activated protein kinase (MAPK) [22].

On the other hand, study on the plant lectin showed lectin from *Pholiotia adiposa* had antiproliferative activity toward hepatoma HepG2 cells and breast cancer MCF7 cells. But the inhibitory activities have not been determined [23].

In this study, we have demonstrated that ether extract of *D. stramonium* inhibited proliferation of cancer cells. Further studies are needed to determine the precise pathway for antiproliferative action.

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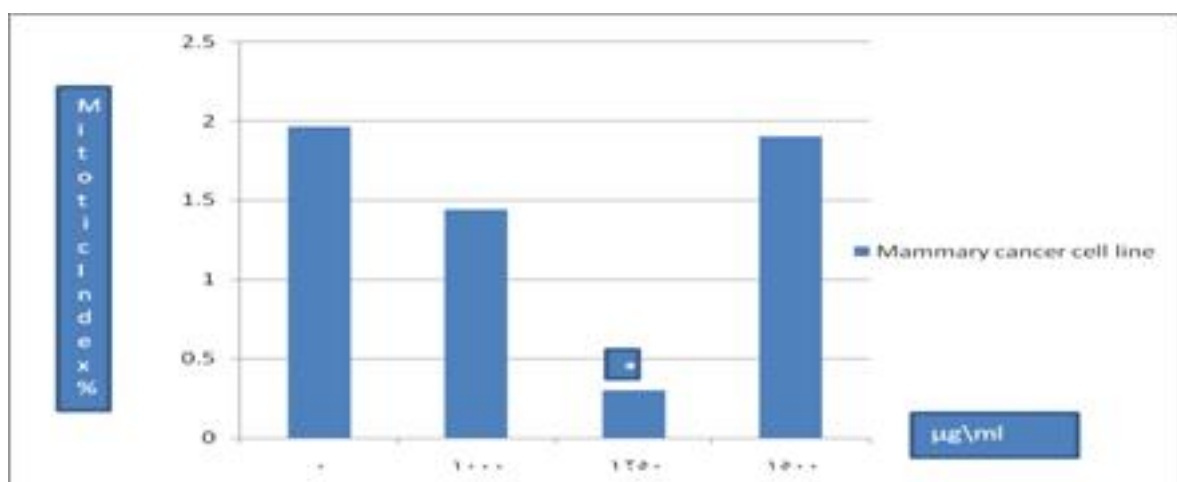


Fig. (1) Mitotic index of mammary cancer cell line after exposure to different concentrations of the ether extract of *Datura stramonium*.

*. Means There is a significant difference at level P

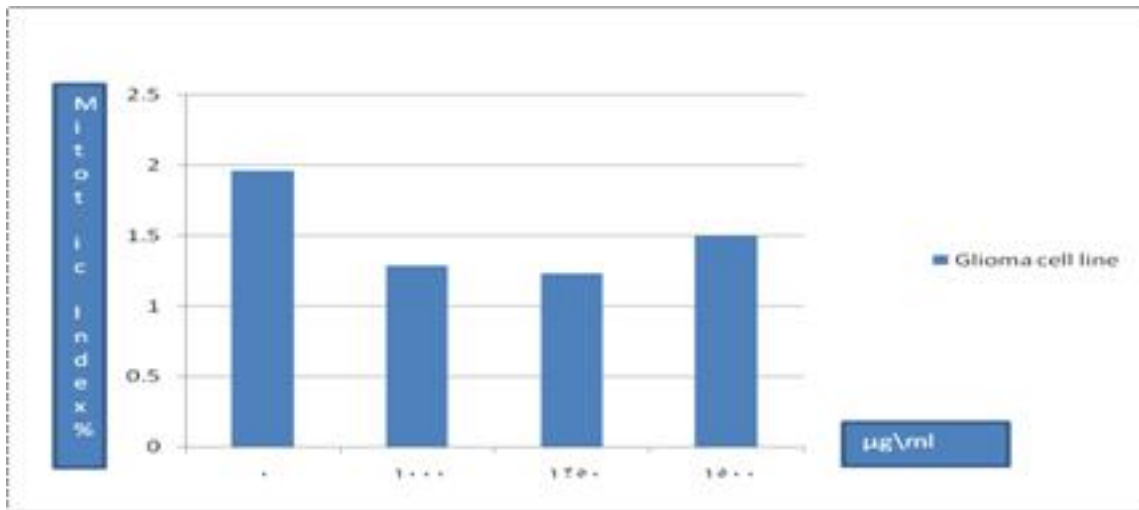


Fig. (2) Mitotic index of glioma cell line after exposure to different concentrations of the ether extract of *Datura stramonium*.

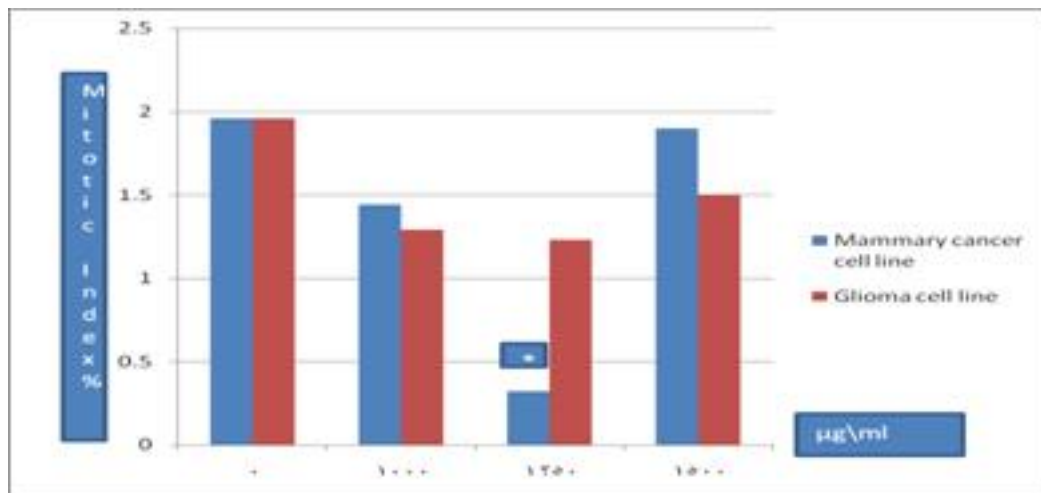


Fig. (3) Comparing between mammary cancer cell line and glioma cell line in mitotic index after exposure to the ether extract of *Datura stramonium*.

تثبيط انقسام الخلايا السرطانية بمستخلص الداتورة سترامونيوم

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الخلاصة

تهدف هذه الدراسة إلى تقييم فعالية مستخلص الداتورة الاثيري في الخلايا السرطانية. تتجه الدراسات الحديثة باتجاه استعمال مواد تعمل بطريقة ما في الحد على إحداث عملية الموت المبرمج أو لمنع الخلايا من الانقسام غير الطبيعي وبهذا توفر ستراتيجية لعلاج السرطان، إذ تتجه بعض الدراسات والبحوث إلى دراسة أنواع مختلفة من النباتات، مثل الفواكه، والخضر، والنباتات الأخرى الصالحة للأكل منها والسامة في الزجاج وفي الجسم إلا أن الدراسات قليلة حول تأثير نبات الداتورة في الخلايا السرطانية ولاتوجد دراسات حول تأثيرها في عملية الانقسام وتكاثر الخلايا السرطانية.

تتضمن هذه الدراسة نوعين من خطوط الخلايا الورمية الخبيثة المزروعة التي تتمثل بخط مزرعة خلايا الغدة البنينة وكذلك خط مزرعة سرطان الدماغ والتي تم تعريضها إلى تراكيز مختلفة من مستخلص الداتورة الاثيري وهي 1000، 1250، 1500 مايكروغرام/ملتر ومن ثم تم تقييم النسبة المئوية لانقسام الخلايا.

أظهرت الدراسة أن هناك انخفاضاً في مؤشرات الانقسام في كلا النوعين للخلايا السرطانية إذ كان الانخفاض معنوياً في سرطان الغدة البنينة وفي مزرعة سرطان الدماغ.

من أهم الاستنتاجات التي توصلت إليها الدراسة أن المستخلص الاثيري لنبات الداتورة يمتلك القدرة على تثبيط انقسام الخلايا السرطانية.

الكلمات المفتاحية: داتورة سترامونيوم، مضادات الانقسام الخلوي، مضادات التكاثر الخلوي.