Inhibition of Mitotic Index and Proliferation of Cell Line H_{22} by Alkaloids Extraction from *Isatis tinctoria*

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Abstract

Alkaloids extraction from *Isatis tinctoria* inhibited H_{22} cell line (hepatic cell line) proliferation by inducing mitotic index at the metaphase/ anaphase boundary. Half-maximal inhibition of cell proliferation occurred at 8 nM alkaloids, and mitosis was half-maximally block 8 nM alkaloids. Formation of an incomplete metaphase plate of chromosomes was associated with inhibition of mitosis and an altered arrangement of spindle microtubules that strongly resembled the organization abnormal that occurs with little concentrations of alkaloids extraction of plant and other antimitotic compounds. No increase in spindle microtubule occurred below 10 nM alkaloids extract. The results indicate that alkaloids shares a common antiproliferative mechanism with extraction alkaloids . The mass of spindle microtubules increased half-maximally at 80 nM alkaloids and attained maximal levels at 33 nM alkaloids extract .At submicromolar concentrations, bovine brain tubulin in a manner that resemble suppression suppressed growing and shortening by extract alkaloids for *Isatis tinctoria* at the ends of microtubules reassembled *in vitro*. The alkaloids was concentrated in H_{22} cells several hundredfold. At its lowest effective concentrations, alkaloids appears to block mitosis by stabilizing spindle microtubules kinetically and not by changing the mass of microtubules polymerized.

Keyword: microtubules :*Isatis tinctoria*: cell line.

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بواسطة القلويدات المستخلصة من نبات الوسمة H_{22}تثبيط مؤشر الانقسام وتكاثر الخلايا السرطانية *Isatis tinctoria*

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الخلاصة

القلويدات المستخلصة من نبات الوسمة لها تاثير مثبط لنمو وتكاثر الخلايا السرطانية (خلايا الكبد الفاري) من خلال تثبيط معامل الانقسام الخلوي عند الطور الاستوائي الانفصالي. نصف تثبيط لتكاثر الخلايا يظهر عند التركيز ثمان نانو مول من القلويد كذلك تثبيط الانقسام الخلوي يظهر عند التركيز ثمان نانومول من القلويد. ان ظهور طور من الانقسام الاستوائي الغير المكتمل من الكروموسومات مقترن مع تثبيط الانقسام و ترتيب النبيبات الدقيقة والتي تكون بشكل منظومة غير طبيعية تظهر عند التراكيز القليلة من القلويد المستخلص من النبات ومضادات الانقسام . لا زيادة في النبيبات الدقيقة تظهر عند تركيز عشرة نانومول من المستخلص القلويدي. ودلت النتائج ايضا ان القلويدات توقف نمو وتكاثر الخلايا. وتزداد كمية النبيبات الى النصف عند التركيز ثمانون نانو مول من القلويد وثلاثون نانومول من القلويد .عند استخدام تراكيز بالمايكرمول من القلويد المستخلص من نبات الوسمة لوحظ كبح نمو وتقصر في نهايات النبيبات الدقيقة.تم اعطاء تراكيز متعددة من القلويد على الخلايا الكبدية السرطانية. وعند التراكيز القليلة من المستخلص القلويدي اظهرت النتائج ان للقلويد فعالية في وقف الانقسام الخلوي من خلال تثبيت حركة نبيبات خيوط المغزل وكذلك يعمل على عدم تغيير في كمية النبيبات الدقيقة المتبلورة. .

الكلمات المفتاحية :النبيبات الدقيقة : نبات الوسمة :خلايا السرطانية

Introduction

Vanblastin and Taxol and many other alkaloids are antimitoticand antitumor heave undergone extensive clinical development as a result of its efficacy in the treatment of refractory breast cancer and its potential value for the treatment of lung cancer, and other cancers (1). The mechanism of action of alkaloids extract Taxol has been considered to be unique.The target for alkaloids appears to be spindle microtubules, and in contrast to,

Colchicine Vinblastine. compounds that can inhibit polymerization microtubule both in *vitro* and *in vivo*, taxol can enhance microtubule polymerization (2, 3). Alkaloids can block mitosis,by induce extensive formation of microtubule bundles in cells, and induce multinucleation of cells during phase of interphase (4-6).It has been suggested that microtubule polymerization responsible for the antitumor activity of the drug. Vincristine and Vinblastine inhibit cell proliferation and mitosis in HeLa cells line without decrease the mass of microtubules and with only subtle changes in the organization of the mitotic spindles.(7-9) also found that low concentrations of alkalois vinblastine inhibit treadmilling dynamic and instability of reassembled bovine brain microtubules in *vitro* without appreciably affecting the microtubule polymer mass (10, 11). *Isatis tinctoria* is a plant genus of brassicaceae family mainly distrbuted in north of Iraq (12) Crude Tryptanthrin alkaloids Indol and indirubin compound found in *I. tinctoria* has undergone for anti- cancer activity (13). On the contrary to the species *Isatis tinctoria* which is understood to contain alkaloids that appear nontoxic in animal studies and have potent tumor-inhibitory effects and anti-angio genetic effects (14). The alkaloids of the species are not yet investigated. This study is the first to assess the activity of locally harvested *Isatis tinctoria* alkaloids against the microtubules of aggressive mice cell line H_{22}, and induction mitoic index.

Materials and Methods

Figure 1: *Isatis tinctoria*

The plant

The plant used in these experiments was gathered from in Erbil (sherwan city) (Figure 1). Plant specimens (leaves, and flowers) identified by botanist doctor Khazal D. Wady ,*Isatis tinctoria* Family Brassicaceae

Alkaloid extraction

Crude alkaloids extraction from the leaves of this plant was extracted as described by (15).and the concentration (0.3,1,3,6,10,33,100)nM.and the period 20 hours .

Cell line

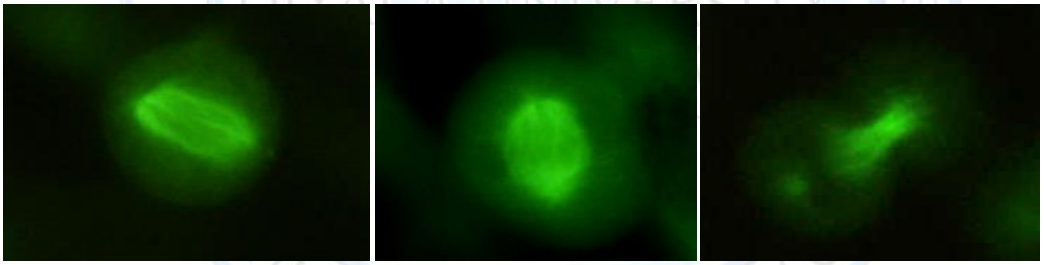
In our experiments we used the mouse H_{22} cell line. This cell line was obtained from the Department of Biology,Faculty of Medicine, Wuhan University, China. were grown at 37°C in monolayers without antibiotics in 5% C02/95% air (14). Cell proliferation was determined by countingcells by hemocytometer at the time of extraction alkaloids addition and 20 h later. Cell morphology, and Mitotic index, and spindle interpolar distances were determined by microscopy immunofluorescence (16).

Used analysis Duncan's multiple test and Complete randomizd Design(CRD).

Results

Relationship of Inhibition of Cell Proliferation, enhancement of microtubule Polymer and mitotic block levels by extraction alkaloids from *Isatis tinctoria* .H_{22} cells were incubated for the duration of one cell cycle with alkaloids over a broad range of concentrations. After 20 h ,cytoskeletons were isolated to determine the mass of tubulinin microtubulin. In parallel experiments, inhibition of proliferation and mitotic index were determined. Alkaloid extract inhibited cell proliferation half-maximally at a concentration of 8 nM, and inhibition was complete at concentrations >33 nM(Fig. 2A). Crude extract alkaloids induced the accumulation of cells in mitotic metaphase half-maximally at a concentration of 8 nM, and maximal mitotic accumulation (80-95%) occurred at alkaloids concentrations of 33 nM and above. Thus accumulation mitotic occurred in parallel with inhibition of proliferation. No

increase in polymer microtubule mass occurred at <10 nM alkaloid. The mass of microtubules then increased as the alkaloids concentration was raised, attaining a half-maximal increase at a concentration of 80 nM and a maximal increase of 500% of the normal level at 330 nM alkaloids .Cells accumulated in metaphase or in a metaphase-like configuration, but no cells were in anaphase either after a 20-h incubation with 10 nM alkaloid (Table 1) or during long-term incubation (as long as 48 h) with 10 nM alkaloid (data not shown). Thus accumulation in mitosis represents a sustained block at the metaphase/anaphase boundary. Of the cells that were in interphase at low alkaloid concentrations (3-10nM), a large percentage (31-38%) consisted of cells with two, three, or more nuclei (Table 1). These results suggest that the mitotic block induced by low alkaloid concentrations is not as sustained as at higher alkaloid concentrations; some cells can escape from mitotic block and become abnormal multinucleated cell.



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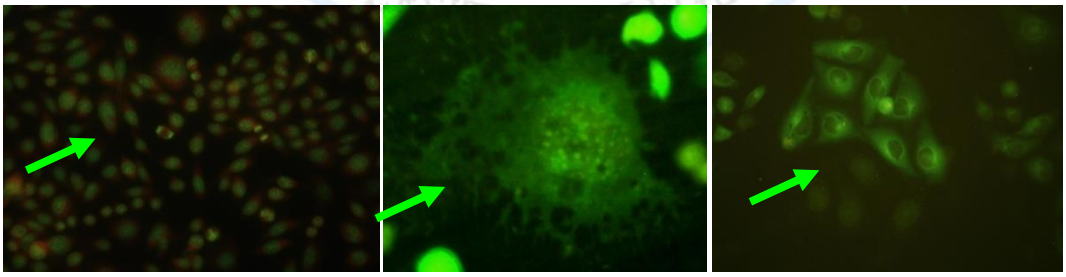

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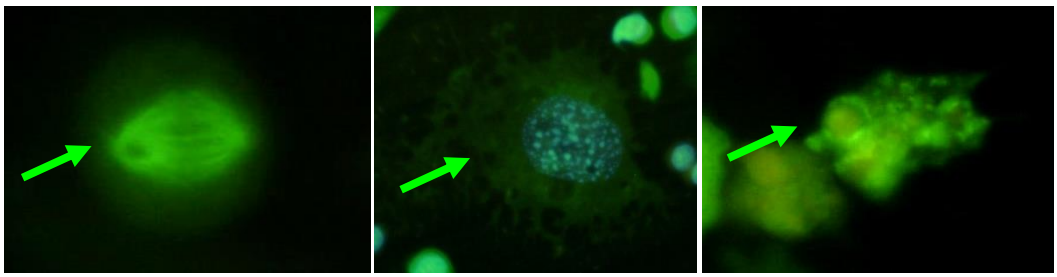


c f i

Figure. 2. Microtubules (a, d,and g), chromosomes (b, e, and h), and centrosomes (c,f, and i) of H_{22} cell mitotic spindles after incubation for 18-20 h with alkaloid.(a-c) Control cell spindle with few astral microtubules and a well defined compact metaphase plate of chromosomes. (d-f) At 6 nM alkaloids, an abnormal bipolar spindle(type I) with prominent astral microtubules (arrow in d) and chromosomes near the spindle poles (arrows in e). (g-i) At 1 ,uM alkaloids, a ball-shaped chromosomal mass with a monopolar microtubule and centrosome arrangement .

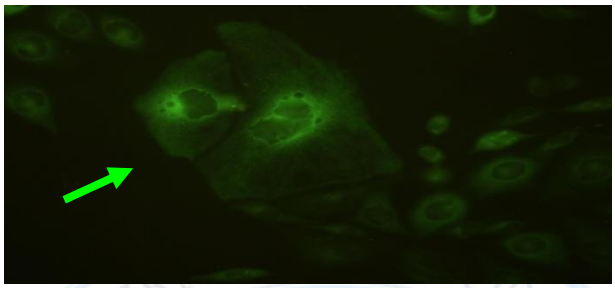
The arrangements of microtubules chromosomes, and centrosomes of control cells and of cells incubated with alkaloids are shown in Fig. 3.Mitotic spindles of cells blocked in metaphase by low concentrations of alkaloids strongly resembled spindles of cells blocked by low concentrations of other antimitotic drugs including vinblastine, vincristine, colchicine, and podophyllotoxin (7, 8) Other spindles (20-32% with 0.33-10 nM alkaloids) were blocked in a nearly normal configuration; these spindles were bipolar with a compact .

Table 1. Effects of alkaloids (20-h incubation) on metaphase/anaphase transition,

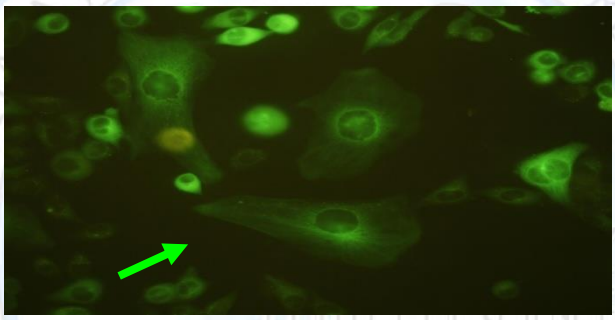
Alkaloid con. nM	Cells in anaphase/ , cells in metaphase,	Multinucleated interphase cells, %
0	0.14 ± 0.03	2.7 ± 0.7
0.3	0.09 ± 0.04	3.2 ± 1.2
1	0.11 ± 0.05	11.6 ± 4.0
3	0.04 ± 0.04	31.2 ± 17.9
6	0.007 ± 0.004	32.6 ± 2.6
10	0	37.7 ±11.5
33	0	16.5± 4.0
100	0	14.0 ± 6.8

Cells in metaphase metaphase plate of chromosomes but with some chromosomes located near the spindle poles (Fig2 d-J) (Table 1) With increasing concentrations of alkaloids, spindle morphology became more abnormal; increasing numbers of chromosomes were located near the poles of bipolar spindles rather than in the metaphase plate. Also as the alkaloids concentration was increased, many spindles had no bipolar organization but were ball-shaped aggregations of condensed chromosomes containing one or more asters of microtubules (Fig. 2 g-i). The morphological changes in spindle structure induced by alkaloids were nearly identical to those that occurred with other antimitotic drugs (7, 8). However, there were some minor differences. Some bipolar spindles blocked by low concentrations of alkaloids appeared to have reduced numbers of interpolar microtubules in contrast with the bipolar spindles induced by low concentrations of other antimitotic drugs that appeared to have normal numbers of interpolar microtubules. Microtubule bundling. Was not when alkaloid concentration ranged between (1-10 nM). However, with 10 nM alkaloids, microtubules often became oriented in parallel fashion. (Compare the meshwork of microtubules of a control cell in interphase in (Fig. 3a) with the array of parallel microtubules radiating out from the nucleus after incubation with 10 nM alkaloids in(Fig. 3b.) Loosely packed bundles of microtubules were observed in a few cells incubated with 33 nM alkaloids

(Fig. 3c), a concentration at which the microtubule polymer mass was double that of control cells.Massive bundles of microtubules formed at higher alkaloids concentrations (e.g., Fig. 3d).



a



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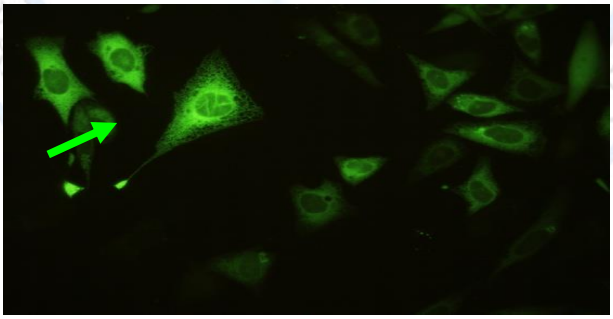

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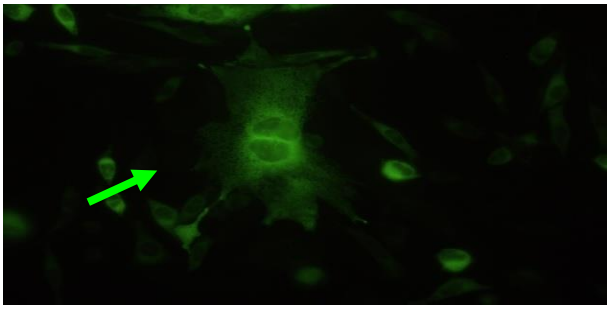

d

Figure. 3. Microtubules of H_{22} cells in interphase incubated for 18-20 h with alkaloids. (a) Control irregular meshwork of microtubules. (b) At 10 nM alkaloids, a somewhat parallel alignment of microtubules but absence of microtubule bundles. (c) At 33 nM alkaloids, a loosely packed bundle of microtubules. (d) At 1 ,uM alkaloids, three compact bundles of microtubules.

Discussion

Very low concentrations of alkaloids are sufficient to inhibit proliferation of H_{22} cells. Both half-maximal inhibition of proliferation and 50% blockage in mitotic index metaphase occurred at 8 nM alkaloids. The degree of metaphase block by alkaloids extract of plant paralleled the degree of inhibition of cell proliferation at all alkaloids concentrations. This observation with alkaloids is consistent with the finding that addition of 10-20 M alkaloid to PtKl cells in early anaphase caused the disappearance of most interzonal microtubules within 5 min (17). In addition,in the present work upon incubation with 10-100 nM alkaloid,some mitotic asters contained no centrosomes (data not shown). Similar results were obtained in PtK2 cells after incubation with micromolar concentrations of alkaloids (18).

Inhibition was associated with formation of an incomplete metaphase plate of chromosomes and an arrangement of spindle microtubules that strongly resembled the abnormal organization that occurs with low concentrations of vinblastine and other antimitotic drugs (19). The most sensitive inhibitory effects of alkaloids extract on proliferation were not

associated with an increase in microtubule polymer mass or with the formation of bundles microtubule, actions of alkaloids that occur at relatively high drug concentrations. No increase in microtubule polymer mass occurred below a alkaloids concentration of 10 nM (Fig. 2 A), and 80 nM alkaloids extract from *Isatis tinctoria* was required to induce a half-maximal increase in the polymer microtubule mass. Thus these results indicate that the most sensitive action of alkaloids extract on H_{22}(hepatic cell line) cell proliferation involves blockage of cell cycle progression at the metaphase/anaphase transition in the presence of a normal mass of microtubule polymer.Phytochemicals such as alkaloids compounds elicit various biological effects including cancer chemoprevention and treatment (20). Some research studied the effect and action mechanisms of try ptanthrin on murine myloid leukemia cells interaction of alkaloids with tubulin, and compared alkaloid and aqueous extract from leaves of *I.tinctoria*(21). Treatment of hippocampus neurons with alkaloid compounds eliminated the microtubule bundles, leaving only tubulin paracrystals. Within 24 hours after washing out the alkaloid, the microtubule bundles repolymerised in cultured cells (22).

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