Cytotoxicity of Two Species of *Glaucium* from Iran

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Abstract

Background: Numerous molecules in Papaveraceae family display interesting cytotoxic activities against tumor cell lines *in vitro* and hints of anticancer activities *in vivo* have been reported in a few cases.

Objective: Numerous molecules in this family display interesting cytotoxic activities against tumor cell lines *in vitro* and hints of anticancer activities *in vivo* have been reported in a few cases. In this study we evaluated the cytotoxic effects of total and alkaloid extracts of *Glaucium flavum Crantz* and *Glaucium grandiflurom Boiss. & Huet*, the two species of this genus, on cell proliferation of HT-29, Caco-2, T47D, and NIH/3T3 cell lines by MTT method and their IC_{50}s were determined.

Methods: The aerial parts of *G. grandiflurom* and *G. flavum* were collected from Jajrud in Tehran Province in June 2011. The effect of total extract and alkaloid extract of them on HT-29, Ta7D, NIH/3T3 and Caco-2 cells was determined by MTT assay.

Results: Alkaloid extracts showed a moderate cytotoxic effect on the cell lines. IC_{50} values confirmed that the growth and proliferation of NIH/3T3 cells were less affected in comparison to other cell lines.

Conclusion: The effects of alkaloid extracts of both plants on human colon adenocarcinoma cell lines (HT-29, Caco-2), showed that these extracts contain certain compounds that can inhibit the proliferation of colon cancerous cells.

Keywords: Cytotoxicity, *Glaucium flavum*, *Glaucium grandiflurom*, MTT

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Introduction

The Papaveraceae family is one of the most important alkaloid containing families. It contains isoquinoline alkaloids including aporphines, protopines, protoberberines and proaporphines [1]. The genus *Glaucium* (Papaveraceae) comprises about 25 species of herbaceous plants in the world and Aporphinoids are the greatest number of isolated alkaloids from this genus [2].

In the past three decades, cancer has been the most important challenge of medicine that can affect approximately 200 types of cells [3]. Cancer is defined as diseases that are characterized by uncontrolled growth and spread of abnormal cells [4]. Use of anticancer drugs produces high rates of cure but some types of cancers are barely affected by currently available drugs. The treatment of cancer has always been a challenging, yet interesting area of research in medicine. The main difficulties faced include drug resistance, toxicity, and low specificity.

Natural compounds and their semi-synthetic and synthetic derivatives are important sources of antitumor drugs. The most promising herbal molecules include the *Vinca* alkaloids; vinblastine, vincristine and vinorelbine, the lignans derivatives; (podophyllotoxines); etoposide and teniposide, the camptothecins; topotecan and irinotecan, and the taxanes; paclitaxel and docetaxel [5].

The genus *Glaucium* (Papaveraceae) comprises about 25 species of annual, biennial or perennial herbaceous flowering plants in the world [6]. In Iran, There are 11 species of *Glaucium* of which, *Glaucium calycinum Boiss.* and *Glaucium contortuplicatum Boiss.*, are endemic [7]. In this study we evaluated the cytotoxic effects of the total and alkaloid extracts of *Glaucium flavum Crantz* and *Glaucium grandiflurom Boiss. & Huet.*

Alkaloids make up an important group of medications to treat cancer. Aporphine alkaloids are the greatest number of isolated alkaloids from the genus *Glaucium.* These alkaloids possess a wide variety of pharmacological effects, including Antiplatelet, antioxidative, antitussive, antiparkinsonic, hypotensive, antiviral, antibacterial, cytotoxic activities [8]. Numerous molecules in this family display interesting cytotoxic activities against tumor cell lines *in vitro* and hints of anticancer activities *in vivo* have been reported in a few cases.

Glaucium grandiflorum is a perennial herb indigenous to various regions of the Middle East extending from the Eastern Mediterranean to Iran. The plant, height 30 – 50 cm, blooms from May until the end of July, its petals is usually dark orange to crimson, with a darker spot at the base [9, 10]. *Glaucium flavum* is a perennial herb which is native to Northern Africa, temperate zones in Western Asia, as well as Europe and is also indigenous to Iran. It is in flower from June to August, and the seeds ripen from August to September. The thick, leathery deeply segmented, wavy, bluish-grey leaves are coated in a layer of water retaining wax [11].

In traditional medicine, the different spieces of genus *Glaucium* were used as laxative, sedative, anti diabetic and anti dermatitis agants [12]. However, no investigation has been carried out on the cytotoxic activity of the plant extracts. The aim of this study was to determine the cytotoxic activity of the total and alkaloid extracts obtained from the aerial parts of the plants.

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Material and Methods

Plant material

The aerial parts of *G. grandiflurom* and *G. flavum* were collected from Jajrud in Tehran Province in June 2011. The plants were identified and authenticated by Dr. F. Salimpour., Faculty of Pharmacy, Azad University of Medical Sciences, Tehran, Iran.

Tested material

Ground dried aerial parts of the plant (500 g each) were separately extracted with MeOH–H_2O (80:20) at room temperature. The procedure was repeated until negative test against Dragendorff's reagent. The MeOH extracts were concentrated to give 11g of *G. flavum* and 8.5g of *G. grandiflurom* crude extracts (yield: 0.022% and 0.017% respectively). 2g of each crude extract were kept for cytotoxicity assays and further studies and the rest were separately dissolved in $CHCl_3$ (500ml) and extracted with 2N HCl (200ml × 10). The acidic fraction was washed with $CHCl_3$ (200ml × 3). The pH of the aqueous solution was adjusted to 2. The combined acid fraction was basified with 25% NH_3 on the ice chest (pH 10-12) and extracted with $CHCl_3$ (300ml × 10). $CHCl_3$ was removed under reduced pressure to give 2.68g of *G. flavum* and 1.54g of *G. grandiflurom* alkaloid extracts. These alkaloid extracts along with the total crude extract s (2 g) were prepared for further studies.

Cell culture

The colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2), and breast ductal carcinoma (T47D) cell lines were maintained as exponentially growing cultures in RPMI 1640 cell culture medium (PAA, Germany) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) for HT-29 cells and 15% FBS for Caco-2 and T47D cells. The Swiss mouse embryo fibroblast (NIH/3T3) cell line was kept in Dulbecco's modified Eagle's medium (DMEM; PAA, Germany) supplemented with 10% FBS. 100 IU/mL penicillin and 100 mcg/mL streptomycin (Boehringer, Germany) were added to the media. All cell lines were cultured at 37°C in an air/carbon dioxide (95:5) atmosphere.

Determination of cell viability by MTT

All the samples, including methanolic and total alkaloid extracts, were tested at 1, 10, 100, 200, 400 and 800 µg/mL concentrations. The samples were dissolved in dimethylsulfoxide (DMSO) and further diluted with cell culture medium. The DMSO final concentration was adjusted to 2% of the total volume of medium in all treatments, including the blank. A control without DMSO was also incubated.

For MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, 1×10^4 cells/well were plated into 96-well plates (Nunc, Denmark) and incubated for 48 h before the addition of drugs. After 48 h of incubation for HT-29 cells, T47D, NIH/3T3 cells, and Caco-2 cells, 20 µL of MTT (Merck, Germany) reagent (5 mg/mL) in phosphate buffered saline (PBS) was added to each well. The plates were incubated at 37°C for 4 h. At the end of the incubation period, the medium was removed and pure DMSO (100 µL) was added to each well. The metabolized MTT product was quantified by reading the absorbance at 570 nm on a microplate reader (Anthos, Austria) [13].

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The cell viability in MTT assay was calculated as a percentage of the control value (untreated cells). IC_{50} (The median growth inhibitory concentration) values were calculated from the IC_{50} of dose–response curve in the SigmaPlot 11 software.

Statistical analysis

All data are articulated as mean ± standard error of the mean. Groups of data were compared with one way analysis of variance followed by Tukey's post hoc test. Data were measured statistically significant when $p < 0.05$.

Results

The IC_{50} values presented in table 1; showed that in HT-29 and Caco2 cell lines, the total extracts of *G. flavum* and *G. grandiflurom* were not significantly different from control group ($p>0.05$) but the alkaloid extracts of both plants were different from control group ($p<0.05$). In T47D cell line, the total extracts of both species did not show cytotoxic effects ($p>0.05$) whereas the alkaloid extracts were slightly different from control group had a weak effect ($p<0.05$).

In NIH3T3 cell line, none of the samples present any cytotoxic effect. The cytotoxic effect of the fractions on NIH/3T3 cells (as nonneoplastic cells) was weaker in comparison to other cell lines. This can suggest that these compounds may be less toxic for the non cancerous cell lines (Figure 1).

Discussion

Aporphinoids form an important group of plant secondary metabolites. Some of these compounds are used for a long time in traditional medicine for the treatment of various diseases, from benign syndromes to more severe illnesses. More than 500 aporphine alkaloids have been isolated from various plant families and many of these compounds display potent cytotoxic activities which may be exploited for the design of anticancer agents. In our study, we examined

Table 1- Cytotoxic activity of total and alkaloid extracts of *Glaucium flavum* and *Glaucium grandiflurom*

Sample[b]	Cell lines[a]			
	HT - 29	Caco - 2	T47D	NIH/3T3
Total extract of *G. f*	348.02 ± 3.51	178.23 ± 41.01	>1000	>1000
Alkaloid extract of *G.f*	22.32 ± 1.34	52.38 ± 3.01	59.48 ± 2.14	170.93 ± 14.67
Total extract of *G. g*	233.49 ± 8.74	133.29 ± 6.98	>1000	203.23 ± 12.1
Alkaloid extract of *G.g*	29.81 ± 1.05	41.38 ± 9.12	56.18 ± 7.17	112.1 ± 0.84

[a]Results are expressed as IC_{50} value (µg/mL), mean of three determinations. Key to cell lines employed: HT-29 and Caco-2 (colon adenocarcinoma), T47D (breast carcinoma), NIH/3T3 (Swiss embryo fibroblast).
[b] One way analysis of variance showed that all test groups were drastically different from control group ($p <0.05$).

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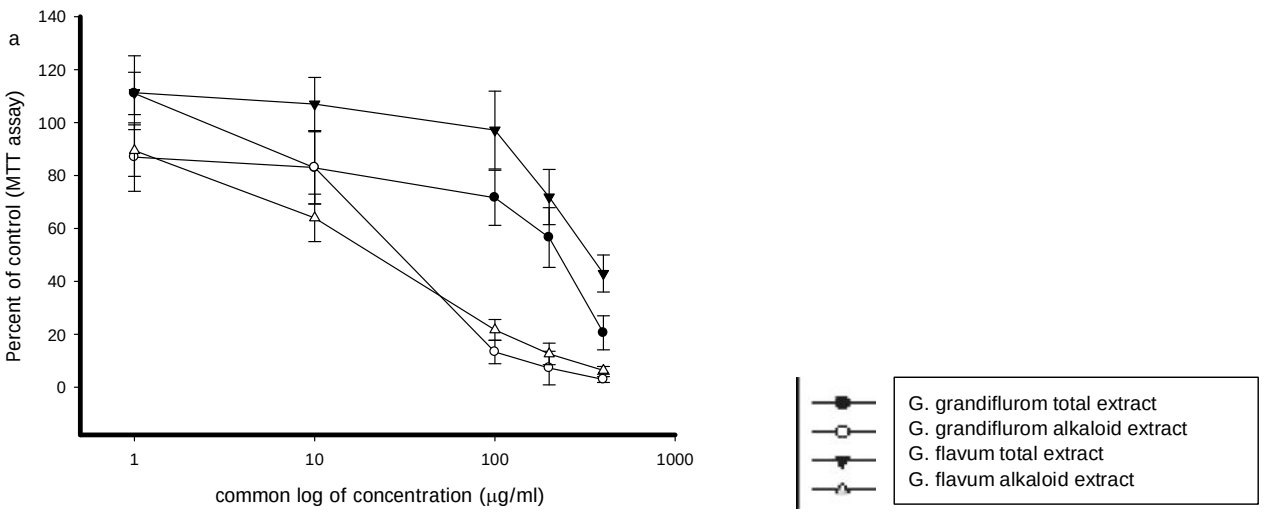

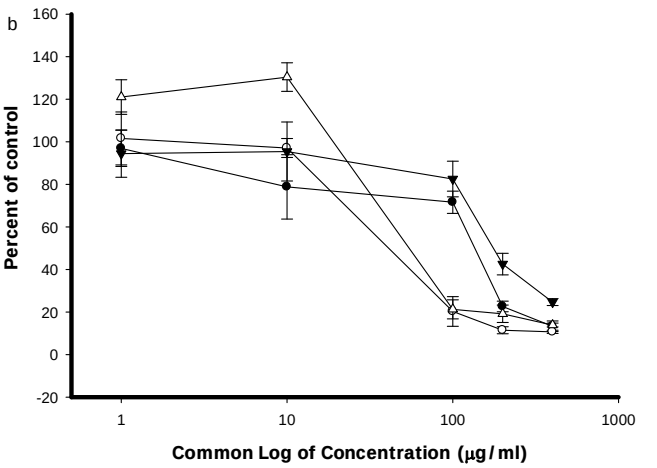

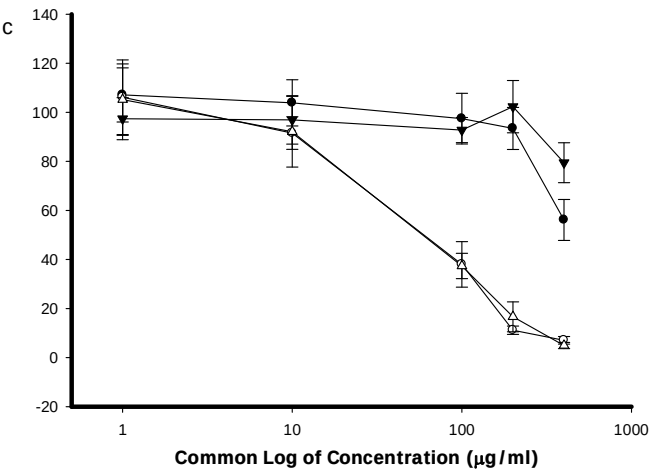

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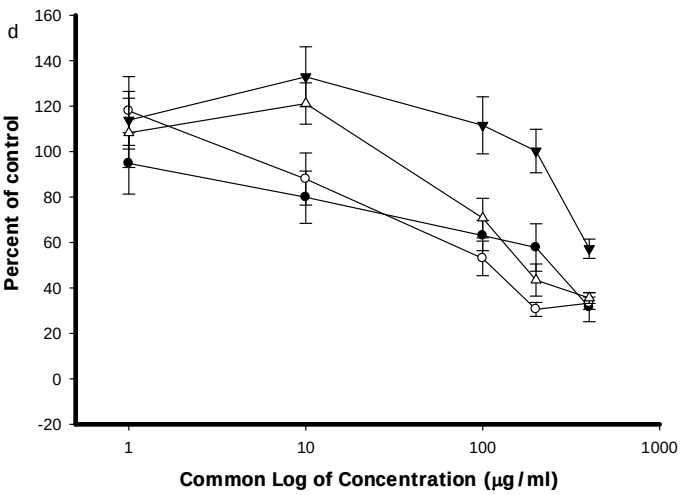

Figure 1. The effect of total extract and alkaloid extract of *Glaucium flavum* and *Glaucium grandiflurom* on cell growth and survival. Cells (1×10^4cells/well) were cultured in the absence or presence of various concentrations of alkaloid extracts, as indicated. After the indicated time points, cell viability was determined by MTT assay. (a) HT-29, (b) Caco-2, (c) T47D, (d) NIH/3T3 cells. The results are presented as mean ± SD.

the cytotoxic effects of two species of the genus *Glaucium*, which are rich in aporphine alkaloids. At least 18 different alkaloids, with a very similar aporphinoid structure, are reported in *G. flavum* [14]. Glaucine, corydine, isocorydine, norisocorydine and isoboldin are some aporphine alkaloids found in both *G. flavum* and *G. grandiflurom* [15, 16].

Although it is clear that aporphines represent an interesting, potentially useful category of anticancer agents, their development is seriously limited by a lack of solid knowledge of their mechanism (s) of action. Different molecular activities have been described, mostly at the nucleic acids level, but overall, the exact targets of these compounds remain elusive. Some of them, like thaliblastine, may be considered as multidrug resistance reversal agents interfering with the Pglycoprotein. Other compounds rather function as topoisomerase inhibitors. This is the case for dicentrinone and dicentrine, but this inhibitory activity is not at the origin of the potent cytotoxicity measured with numerous aporphines (Stévigny, 2005).

The effects of alkaloid extracts of both plants on human colon adenocarcinoma cell lines (HT-29, Caco-2), showed that these extracts contain certain compounds that can inhibit the proliferation of colon cancerous cells.

The absence of cytotoxic effect in some extracts could be for the reason of impurity of the extracts. Isolation and characterization of the alkaloid structures as well as investigation of the specific cytotoxic pathway may help to determine whether these extracts are valuable for antineoplastic effects.

Conclusion

The strongest effects of the total extracts of *Glaucium flavum* and *Glaucium grandiflurom*, were on Caco2 cell line (IC_{50} around 178 µM for *G. flavum* and IC_{50} around 133 µM for *G. grandiflurom*). The best cytotoxic effect of the alkaloid extract of *Glaucium flavum*, was

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on HT29 cell line (IC_{50} around 22 µM) and this effect was the strongest cytotoxic effect observed in this study. The strongest cytotoxic effect of the total extract of *Glaucium grandiflurom*, was on HT29 cell line as well (IC_{50} around 30 µM).

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