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IN VITRO ANTICANCER ACTIVITY AND CYTOTOXICITY OF SOME PAPAVER ALKALOIDS ON CANCER AND NORMAL CELL LINES

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Abstract

Background: The purpose of this study is to determine the effect of *in vitro* anticancer activity and cytotoxicity of 13 *Papaver* alkaloids (amurine, armepavine, berberine, isocorydine, isothebaine, macranthine, mecambrine, mecambridine, narkotine, orientalidine, oripavine, salutaridine and thebaine) against the human cervical cancer cell line (HeLa) compared to the normal African green monkey kidney epithelial cell line (Vero) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
Materials and Methods: The Vero and HeLa cell lines were treated with various concentrations (1-300 µg/mL) of alkaloids for 48 h. Values for cytotoxicity measured by MTT assay were expressed as the concentration that causes a 50% decrease in cell viability (IC_{50}) (µg/mL).
Results: Berberine and macranthine were the most active alkaloids. Salutaridine exhibited no cytotoxic activity against two types of cell lines. Dose-dependent studies presented IC_{50} of 12.08 µg/mL and IC_{50} of 71.14 µg/mL for berberine and IC_{50} of 24.16 µg/mL and IC_{50} of >300 µg/mL for macranthine on the HeLa cells and the Vero cells respectively.
Conclusion: The degree of selectivity of the compounds can be expressed by its Selectivity Index (SI) value. High SI value (>2) of a compound gives a selective toxicity towards cancer cells (SI = IC_{50} for normal cells/IC_{50} for cancer cells). Two alkaloids showed significant SI values, which are 12.42 for macranthine and 5.89 for berberine. Hence, macranthine and berberine display potential to be further exploited in the discovery and development of new anticancer agents.

Key words: Cytotoxicity, Anticancer activity, Papaver alkaloids, HeLa cell line, Vero cell line, MTT assay

Introduction

Plant extracts are useful sources of new medicines; thereby finding applications in the pharmaceutical industry. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment (Dwivedi et al. 2013). The use of medicinal plant extracts for the treatment of human diseases is an ancient practice and this has greatly increased in recent years (Khakdan & Piri 2013). Cancer is one of the most life-threatening diseases with more than 200 different types. Due to lack of effective drugs, expensive cost of chemotherapeutic agents and their side effects, cancer can be a cause of death (George et al. 2010). Plants have been used in the treatment of cancer for ages. Although, excellent antitumor activities of common chemotherapy drugs treatment will be restricted in some cases due to drug-resistance, low therapeutic index, severe side effects and different routes of administration. There has been an emphasis on herbal and natural compounds in a recent cancer research (Afzali et al. 2015). Research into plants with anticancer effects is still encouraged with a view to discover any new drugs with less toxic but more potent effects. At present, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to (Mahadev et al. 2015).

Plant synthesized many compounds with complex molecular structures as a result of secondary metabolism. Some of the compounds and their derivatives such as alkaloids, flavonods, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds have many medicinal properties (Praveena & Suriyavathana 2014). Among the natural products, the alkaloids, biologically active secondary metabolites that can be found in plants, animals or microorganism, stand out. Biosynthetically, the alkaloids are derived from amino acid biosynthesis or transamination processes, and they are classified according to the amino acid that yields the nitrogen atom as well as the part of its skeleton for the synthesis of the alkaloid in question. Therefore, the alkaloids are compounds consisting of a basic nitrogen atom that may or may not be a part of heterocyclic ring. Alkaloids are endowed with diverse biological activities, being already used in therapy as pharmacological tools. Among the reported biological effects, they present antitumor (Tahme et al. 2011, El Shazly et al 2014), anticholinergic (Berdai et al. 2012), diuretic (Melendez-Camargo et al. 2014), antiviral (Orhana et al. 2007), antihypertensive (Awaad et al. 2007), antidepressant (Nesterova et al. 2011), antimicrobial (Karou et al. 2006), antiemetic (Bulbul et al. 2013), and anti-inflammatory (Vijayalakshmi et al. 2011) properties. Nonetheless, there are also reports of toxic effects to humans; thus, the use of different experimental models to understand the exact mechanism of the molecules under study is necessary, in order to have the real knowledge of their effect (Nascimento et al. 2015). *In vitro* cytotoxicity investigations on plant extracts are commonly the first steps of research for anticancer compounds from natural sources (Erel et al. 2014).

Hence in the present study, an attempt has been made to find out the *in vitro* anticancer and cytotoxic activity of 13 *Papaver* alkaloids against the human cervical cancer cell line (HeLa) compared to the normal African green monkey kidney epithelial (Vero) cell line.

Material and Methods

Preparation of Alkaloids

Alkaloids were obtained from the aerial parts of the *Papaver* species (Table 1) following the reported method (Sariyar et al. 1990; Mat et al. 2000; Sariyar 2002). The extracts of the samples were separated by column chromatography on silica gel eluting with $CHCl_3$ and $CHCl_3$: MeOH (90:10; 80:20). Fractions of 30 mL were collected and similar fractions were combined and separated by preparative thin layer chromatography on silica gel. The identification of the alkaloids was carried out by comparing their physical and spectral data and TLC values with those of authentic samples. List of alkaloids isolated from *Papaver* species shown in Table 1. The alkaloids were dissolved in chloroform. They were then prepared at various concentrations in the medium (Eagle's minimum essential medium).

Cell Cultures

The human cervical cell line (HeLa) and the normal African green monkey kidney epithelial (Vero) cell lines were grown and maintained in Eagle's minimum essential medium (EMEM) with Earle's saline, supplemented with an antibiotic-antimycotic mixture [penicillin (100 U/mL), streptomycin (100 µg/mL), amphotericin B (0.25 µg/mL)], and 10% fetal bovine serum. Cells were maintained in a humidified atmosphere containing 5 % CO_2 at 37°C.

In Vitro Cytotoxicity Assay

We measured the anti-proliferative activity of alkaloids by using MTT assay (Mosmann 1983, Karagöz et al. 2009, Hasibuan 2014, Masriani 2014, Mahadev et al. 2015, Paul et al. 2015). This colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzyme in living cells to reduce the yellow water soluble substrate MTT into an insoluble, colored formazan product, which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The cells were harvested ($2x10^4$cells/well) and inoculated in 96 well plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cells were then inoculated with and without the alkaloid (final alkaloid concentrations are ranged 1-300 µg/mL). After 48 h incubation, the medium was removed. Thirty five µL of MTT solution (5 mg/mL in PBS, pH 7.2) was added to each well and the plates incubated for 4 h at 37 °C. After incubation, 200 µL of dimethyl sulfoxide was added to each well of plates, followed by gentle shaking to solubilize the formazan dye for 15 min. Absorbance was measured at 540 nm and 620 nm using a microplate reader.

The percentage growth inhibition was calculated using following formula:

$$\% \text{ cell inhibition} = 100 - \frac{\text{Absorbance value of alkaloid treated cells}}{\text{Absorbance value of control cells}} \times 100$$

All experiments were performed in triplicate and mean values were used for calculation. Spectrophotometric determinations were performed using µ Quant Universal Microplate Spectrophotometer (Bio-Tek) and data was statistically processed by KCJunior Data Program. IC_{50} value was obtained from dose response curve of percent viability versus test concentrations. IC_{50} calculations were performed by using GraphPad Prism Software.

Table 1: List of alkaloids isolated from *Papaver* species.

Alkaloids	*Papaver* Species
Amurine	*P. apokrinomenon* *P. pilosum* *P. strictum*
Armepavine	*P. fugax* *P. persicum*
Berberine	*P. curviscapum* *P. polychaetum* *P. dubium ssp. dubium* *P. dubium ssp. laevigatum* *P. dubium ssp. lecoqii*
Isocorydine	*P. commutatum ssp. Euxinum* *P. rhopalothece* *P. macrostomum*
Isothebaine	*P. pseudo-orientale*
Macranthine	*P. pseudo-orientale*
Mecambrine	*P. armeniacum* *P. fugax* *P. triniifolium*
Mecambridine	*P. lasiothrix* *P. pseudo-orientale*

Narkotine	*P. cylindricum* *P. fugax*
Orientalidine	*P. pseudo-orientale*
Oripavine	*P. orientale* *P. cylindricum*
Salutaridine	*P. bracteatum* *P. lasiothrix* *P. pseudo-orientale* *P. fugax* *P. persicum*
Thebaine	*P. bracteatum* *P. cylindricum* *P. triniifolium*

Selectivity Index (SI)

The degree of selectivity of the compounds can be expressed by its SI value as suggested by Badisa et al. (2009). High SI value (>2) of a compound gives a selective toxicity towards cancer cells. While the compound with SI value <2 is considered to give general toxicity in which it also can cause cytotoxicity in normal cells (Masriani 2014). Accordingly, each SI value was calculated using the formula given below:

$$SI = IC_{50} \text{ for normal cells} / IC_{50} \text{ for cancer cells}$$

Results and Discussion

The plant kingdom represents an enormous reservoir of biologically active molecules and so far, only small fractions of plants with medicinal activity have been assayed. Nearly 50% of drugs used in medicine are of plant origin. There is therefore much current research devoted to the phytochemical investigation of higher plants that have ethnobotanical information associated with them (Elhardallou 2011). Botanicals such as herbal products and nutraceuticals are often regarded as low risk since they have been used by human throughout history. However, some of them may reveal a very strong and even toxic activity in humans, which especially refers to extracts, concentrates or pure compounds obtained from plants. For this reason, it seems very important to conduct screening tests to assess both the beneficial effects and the toxicity of plant materials (Sieniawska et al. 2013).
In this study, we first examined cytotoxicity and selectivity of 13 *Papaver* alkaloids (amurine, armepavine, isocorydine, isothebaine, macranthine, mecambrine, mecambridine, narkotine, orientalidine, oripavine, salutaridine, thebaine and berberine) on the normal Vero cell and the HeLa cervical cancer cell lines using MTT assay. Results are expressed as IC_{50} and SI value of the normal Vero cell and the HeLa cervical cancer cell line and shown in Table 2. The final concentration of chloroform is lower than 0.1%. There was no toxicity on two cell lines.

Table 2: Cytotoxic activity as expressed as IC_{50} (µg/mL) of *Papaver* alkaloids.

Alkaloids	HeLa IC_{50} (µg/mL)	Vero IC_{50} (µg/mL)	SI*
Amurine	151.51±3.68	86.58±2.11	0.57
Armepavine	66.44±3.32	95.30±1.15	1.43
Berberine	12.08±0.14	71.14±1.59	5.89
Isocorydine	ND	>300	ND
Isothebaine	ND	>300	ND
Macranthine	24.16±0.49	>300	> 12.42
Mecambrine	36.91±1.14	58.39±4.01	1.58
Mecambridine	ND	>300	ND
Narkotine	ND	>300	ND
Orientalidine	200±2.13	>300	>1.5
Oripavine	271.81±6.13	110.74±4.47	0.41
Salutaridine	ND	ND	ND
Thebaine	ND	54.56±1.89	ND

Data are expressed as the means of triplication.
ND: Not determined.
*(SI) Selectivity Index = IC_{50} Vero cell/ IC_{50} HeLa cell.

SI value > 2 indicating high selectivity (Machana et al. 2011, Awang et al. 2014).

From the tested alkaloids, berberine, macranthine, mecambrine for the HeLa cells and thebaine, mecambrine, berberine for the Vero cells showed cytotoxic activity. Whereas, berberine and macranthine showed the highest cytotoxic activity against HeLa cancer cell line but these alkaloids exhibited low cytotoxic activity against the Vero normal cell line. Salutaridine exhibited no cytotoxic activity against two types of cell lines. Mecambrine alkaloid showed 100 % cytotoxic activity on the Vero cells at 100 µg/mL and the HeLa cells at 150 µg/mL concentration.

Two of the 13 tested alkaloids exhibited a substantial anti-proliferative effect on the HeLa cells. The most active alkaloids were berberine and macranthine. Dose-dependent studies revealed IC_{50} of 12.08 µg/mL and IC_{50} of 71.14 µg/mL for berberine and IC_{50} of 24.16 µg/mL and IC_{50} of >300 µg/mL for macranthine on the HeLa cells and the Vero cells respectively. The IC_{50} values were used to determine the selectivity indexes (SI) of each alkaloids which represents the overall activity.

The degree of selectivity of the compounds can be expressed by its Selectivity Index (SI) value. The SI values were calculated as follows: SI = IC_{50} normal cell/IC_{50} cancer cell. High SI value (>2) of a compound gives a selective toxicity towards cancer cells (Badisa et al. 2009). Selectivity of the cytotoxic activity of the 13 tested alkaloids was determined by comparing the cytotoxic activity (IC_{50}) of each alkaloids extract against the cancerous HeLa cell with the normal Vero cell. Two alkaloids showed significant SI values, which are 12.42 for macranthine and 5.89 for berberine. Hence, macranthine and berberine display potential to be further exploited in the discovery and development of new anticancer agents.

Berberine alkaloid from *Papaver* species (*P. curviscapu, P. polychaetum, P. dubium ssp. dubium, P. dubium ssp. laevigatum* and *P. dubium ssp. lecoqii*) (SI=*5.89*) and macranthine alkaloid from *Papaver pseudo-orientale* (SI= >12.42) showed the most promising and selective cytotoxic activity against HeLa cell line. In a previous study, Actinomycin D, an anticancer agent, had an IC_{50} values of 0.002 ± 0.0000395 µg/mL for HeLa cell line and 0.027 ± 0.00021 µg/mL for Vero cell line and its SI value was found 13.5 (Berrington & Lall 2012). In our study, especially the macranthine alkaloid exhibited highest cytotoxic effect on the HeLa cell line, whereas low cytotoxicity on the Vero cell line. Consequently, macranthine alkaloid could be considered as a promising anticancer agent due to its high SI value.

We showed that the alkaloids (especially berberine and macranthine) from some *Papaver* species have significant *in vitro* anticancer activity by the results of the study. Further investigations may lay on additional consideration into how to obtain the cytotoxicity of alkaloids *in vivo* as useful anticancer agents.

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