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# THE CYTOTOXIC EFFECT OF CHELIDONIUM MAJUS IN VITRO

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## ABSTRACT

The effect of aqueous and ether Chelidonium majus haulms extract on cervical HeLa tumor cells, mammary adenocarcinoma MCF 7 tumor cells and acute lymphoblastic leukemia CEM tumor cells in vitro have been studied. The purpose of this research was to compare the effect of aqueous and ether Chelidonium majus haulms extract on selected tumor cells. Colorimetric MTT assay have been used for the study of the antiproliferative effect of aqueous and ether haulms extract of Chelidonium majus on cell viability in vitro. The results of the experiments have shown the cytotoxic effect of the aqueous and the ether Chelidonium majus haulms extract on the individual tumor cells. The aqueous Chelidonium majus haulms extract was the most effective on CEM cells, it was less effective on MCF 7 cells and it was the least effective on HeLa cells. The ether haulms extract of Chelidonium majus was the most effective at all of studied concentrations on CEM cells and MCF 7 cells in comparison with HeLa cells, where it was significantly effective only at the highest concentration. Aqueous and ether haulms extract of Chelidonium majus tested in vitro indicated their cytotoxic activity. Both haulms extract of Chelidonium majus were more efficient on CEM cells. It is assumed that higher antiproliferative activity of ether haulms extract of Chelidonium majus is the result of higher antiproliferative activity of lipophilic substances. The lipophilic substances pass through membrane and bind to various proteins and change their biological activity.

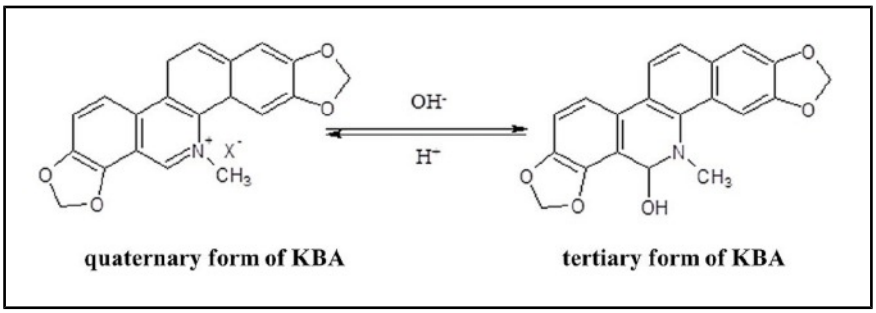
## UDC CODE & KEYWORDS

■ UDC: 615 ■ Cytotoxic effect ■ Chelidonium majus extract ■ MTT assay

## INTRODUCTION

The benefit of medicinal plants is their natural occurrence and lower incidence of adverse effects. Chelidonium majus (greater celandine) contains mixture of different hydrophilic and lipophilic bioactive substances eg alkaloids of the isoquinoline type and derivatives of caffeic acid. The alkaloids are found in the largest amounts in the plant (Minarik, 1979). The content and the ratio of alkaloids in individual parts of plant depends on many factors particularly the vegetation period. The highest content of alkaloids of all of plant organs have been found in the root (Slavik, 1980). The duality of the effect of Chelidonium majus extracts in vitro and in vivo can be assumed according the experimental results from existing scientific literature (Dostal & Slavik, 2000). Important dual property of benzo[c]phenanthridine alkaloids as the main constituent of Chelidonium majus is the reversible conversion of their quaternary structure to tertiary isomer (Figure. 1), depending on the environmental influences as the pH value or polarity of the solvent. Benzo[c]phenanthridine alkaloids can act as natural indicators. Alkaloids with tertiary structure (also called pseudobase) are colorless in alkaline pH range, while in an acidic pH range alkaloids are colored and have a positively charged quaternary nitrogen structure of alkaloid (Malikova, Zdarilova, & Hlobilkova, 2006). Quaternary polar form (KBA) is brightly colored and highly soluble in water—hydrophilic. The tertiary non-polar form (TBA) is colored and highly soluble in lipids – lipophilic (Dostal & Slavik, 2000).

Figure 1: Reversible transformation of alkaloids




Source: Dostal & Slavik (2000)

Bioactivity of benzo[c]phenanthridine alkaloids on cellular and molecular level influences the planarity of aromatic ring, the reactivity of quaternary alkaloid cation and the number, the type and location of substituents (Dostal & Potacek, 1990). Both structures KBA and TBA can be present in different ratio. The ratio between the quaternary and tertiary form decides about the bioactivity of alkaloid. Benzo[c]phenanthridine alkaloid TBA pass through the cell membrane in tertiary lipophilic structure and inside the cell it is converted to quaternary hydrophilic structure KBA, that has an antibacterial, antiviral and cytotoxic effect (Ishikawa, 2001). Berberine and isoquinoline alkaloids showed antihyperglycemic and hypoglycemic effect on diabetic rats, in which these substances inhibited the activity of mitochondria and reduced the amount of ATP within the cell (Xia et al., 2001). Chelidonic acid can bind with the plant alkaloids and it has a mild antibacterial and analgesic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effect in this form (Tomko, 1999). Alkaloids are reactive inside the cell. They can interact with with proteins, DNA and lipids (Ulrichova et al., 2001). The final effect of these molecules is always a combination of different interactions, mainly electrostatic interaction of cation, nucleophilic addition on imine bond and hydrophobic interactions (Vavreckova & Ulrichova, 1994). Scientists pointed out to possible hepatotoxic effect of Chelidonium majus when it was used in higher quantity (Stickel et al., 2003). It is very popular short-term usage of Chelidonium majus in smaller amounts, especially for its cytoprotective, cholagogic and spasmolytic effect in current medicine (Crijns, de Smet, van den Heuvel, Schot, & Haaqsma, 2002), as well as for its anti-inflammatory, anticancer and antibacterial activity (Jiming et al., 2001). Several studies confirmed the cytotoxic effect of Chelidonium majus in the treatment of cancer (Lanvers-Kaminsky et al., 2006). Currently, semisynthetic drug UkrainTM (NSC-631570) which contains the purified alkaloids of Chelidonium majus is used for the treatment of cancer in several countries eg Ukraine, Mexico and others (Boehm & Edzard, 2013).

**Research and purpose**

In the present work it has been studied the possible dual cytoprotective and cytotoxic effect of the aqueous and the ether extract of Chelidonium majus on human tumor cell lines of type HeLa, MCF-7 and CEM in dependence on the concentration of individual extracts of greater celandine by using MTT colorimetric assay.

**Materials and methods**

The cells were provided by Dr. Hajduch (Olomouc, Czech Republic) to the Department of Pharmacology Medical Faculty Košice, UPJŠ Košice, originally purchased from American Type Culture Collection (ATCC). SDS, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany) and ether were purchased from Merck, Germany. The media, antibiotics, special plastic culture vessels for cultivation cells were purchased from Invitrogen, USA. The aqueous and also the ether extract was prepared 21 days before use in a closed container from the finely minced powder (30 g) of dried haulms of Chelidonii herba drug (Agrokarpaty s.r.o., Slovakia), collected during of flowering from May to September, 2013. The powder of dried haulms of Chelidonii herba drug was leached in (300 g) appropriate solvent (redistilled water and ether) for the everyday shaking. The aqueous and ether plant mixture was filtered and from the filtrate under reduced pressure at temperature t = 40˚- 50˚C was obtained the dry aqueous and ether haulms extract of Chelidonium majus. The yield of the aqueous extract was 1.53 g (5.1%) and the ether extract yield was 1.15 g (3.83%). The aqueous and ether haulms extract of Chelidonium majus was gradually diluted with culture medium from the highest concentration $c_1$ to the lowest concentration $c_6$. The ether haulms extract of Chelidonium majus was dissolved in 10 % dimethylsulfoxid (Mosmann 1983), and then it was diluted with culture medium. Tumor cell lines of HeLa and CEM were grown in RPMI 1640 medium containing antibiotics (penicillin 10 000 U/ml and streptomycin 10000 µg/ml) and fetal calf serum (10 %). MCF-7 cells were incubated in DMEM medium with GlutaMAXom, fetal calf serum (10 %) and antibiotics (penicillin 10 000 U/ml a streptomycin 10000 µg/ml). Passaging (14th passage) and incubation of control (without plant extracts) and experimental cells (with aqueous / ether haulms extract of Chelidonium majus) was realized in the thermostat (SANYO CO2 incubator, model MCO-19AIC, Japan) at temperature t = 37 °C, in the environment of air, containing 5% CO2 in 96-well culture plates for 72 hours. Each sample in dimple contained 80 µl of cell suspension and 20 µl of culture medium. Sample with HeLa cells contained 246 400 of cell suspension in 80 µl. Sample with CEM cells contained 353 600 of cell suspension in 80 µl. Sample with MCF-7 cells contained 326 400 of cell suspension in 80 µl. The experiments were carried out in sterile conditions. Working with cells was carried out in a laminar flow box. Cytotoxic effect of extracts of Chelidonium majus on the survival of tumor cell lines in vitro was determined by MTT colorimetric assay using a spectrophotometer (ELISA reader PowerWave HT Microplate Spectrophotometer, BioTek®, USA) at the wavelength λ = 540 nm. MTT and 72 hour incubation of tumor cell lines was performed according to the methodology of Mosmann MTT (Mosmann, 1983). The measured results were processed by analytic software with the program GEN5TM. From results of absorbance (average value ± SD, n = 9) was determined survival of experimental cells (x %) in comparison with the control cells (100 %). The average percentage of survival of different types of tumor cell lines (x %), depending on the effect of different concentrations of the aqueous and the ether haulms extract of Chelidonium majus. Experimental cells were compared statistically to the control cells (100%) by using software GraphPad InSTAT and Tukey-Kramer test. All collected data were processed by one-way ANOVA test. Each experiment, each biological sample was detected three times for a minimization of the variability of biological material. The curves were evaluated with PRISM which calculated the maximum effect ED50% of the individual Chelidonium majus haulms extracts (Table 1).

Table 1: The maximal effect ED50% of the individual Chelidonium majus haulms extracts

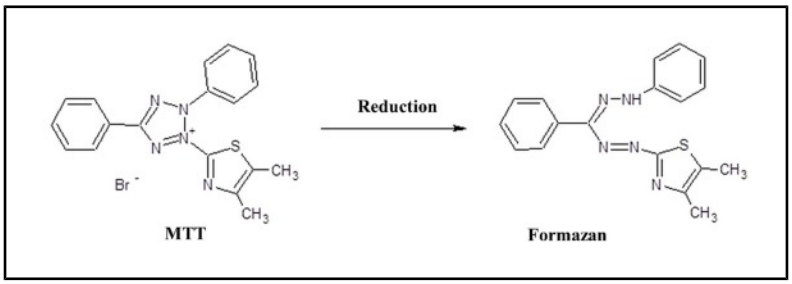
| Extract and cells | Concentration(µg/ml) ED 50 ± SD |
|---|---|
| Aqueous extract of HeLa | 1005.00 ± 10 |
| Ether extract of HeLa | 278.00 ± 14 |
| Aqueous extract of MCF 7 | 435.00 ± 10 |
| Ether extract of MCF 7 | 35.00 ± 7 |
| Aqueous extract of CEM | 138.00 ± 5 |
| Ether extract of CEM | 33.00 ± 8 |

Source: Tomečková et al. (2015)

**Results and discussion**

The principle of the MTT method is to determine (Figure 2) ability to convert (reduce) soluble yellow tetrazolium salt to insoluble violet blue formazan by mitochondrial respiratory enzymes inside living cells (Mosmann, 1983).

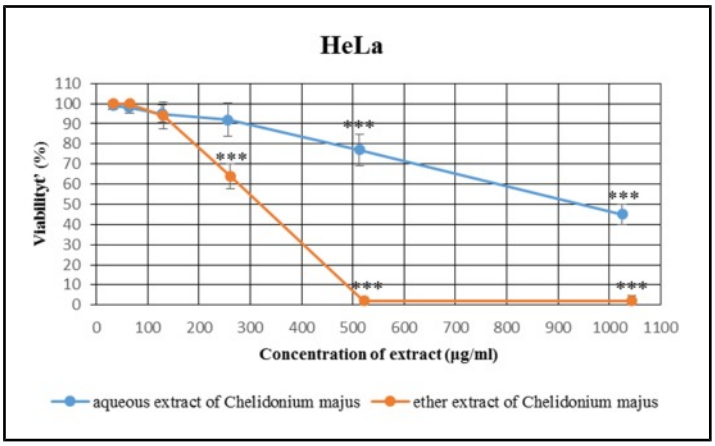
Figure 2: The conversion of 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) to formazan




Source: Mosmann (1983)

Enzymes of mitochondria in apoptic cells do not reduce MTT to formazan, and therefore yellow colour of MTT persists. MTT test is more convenient method such as radioisotopic test. The advantages of the MTT assay are the sensitivity, the reproducibility, the elimination of radioactive components, simplicity and speed evaluation of results (Ferrari et al., 1990). Results of this study showed dependence of cytotoxic effect of aqueous and ether haulms extract of Chelidonium majus on the concentration of used extracts. Ether extract of haulms of Chelidonium majus showed higher cytotoxic effect on HeLa cells in comparison with the aqueous haulms extract of Chelidonium majus (Figure 3). The aqueous extract of haulms of Chelidonium majus was the most effective on CEM cells (Figure 5) and MCF-7 cells (Figure 4).

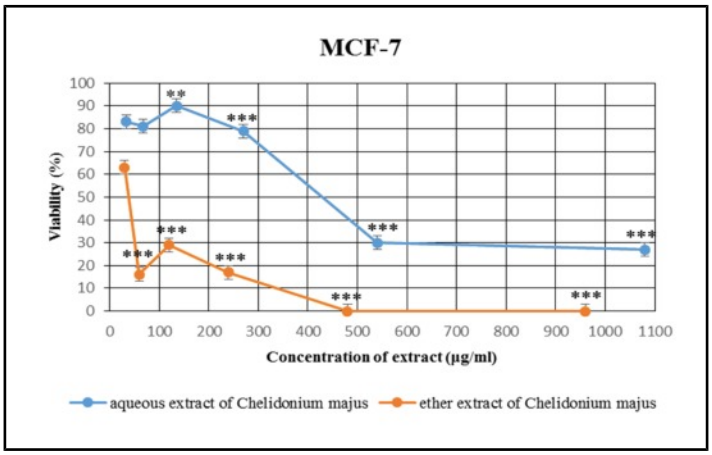
Figure 3: Comparison of the cytotoxic effect of Chelidonium majus haulms extract on HeLa cells, the average lifespan of cells ± SD, n = 9




Source: Tomečková et al. (2015)

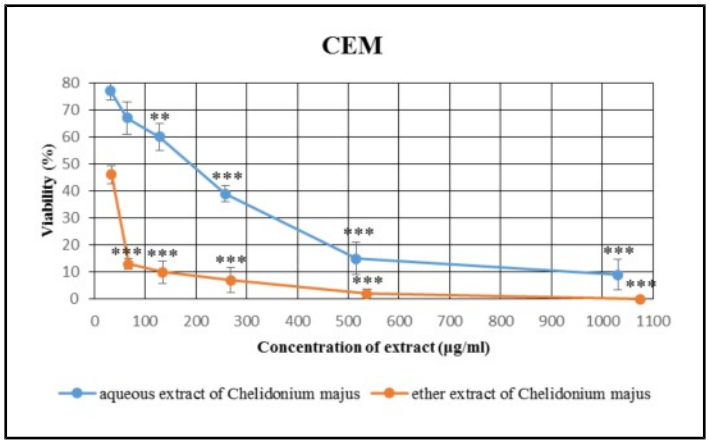
The least efficient it was on HeLa cells (Figure 3). Ether extract of haulms of Chelidonium majus was significantly effective ($p < 0.001$) at all studied concentrations on CEM cells (Figure 5) and MCF–7 cells (Figure 4) in comparison with the HeLa cells, where it was significantly active only at the highest concentration (Figure 3). Most in vitro studies reported that constituents of Chelidonium majus haulms extract are responsible for its anticancer activity. These substances change their structure and the activity in cell depends on the environment as the pH and polarity of the solvent.

Figure 4: Comparison of the cytotoxic effect of Chelidonium majus haulms extract on MCF-7 cells, the average lifespan of cells ± SD, n = 9




Source: Tomečková et al. (2015)

Figure 5: Comparison of the cytotoxic effect of Chelidonium majus haulms extract on CEM cells, the average lifespan of cells ± SD, n = 9




Source: Tomečková et al. (2015)

Benzo[c]phenanthridine alkaloids penetrate the cell membrane in the lipophilic tertiary structure and after intrusion into cell they are converted to hydrophilic quaternary structure. Benzo[c]phenanthridine alkaloids in quaternary structure interact with DNA and are intercalated in DNA molecule. Sanguinarine is the strongest anticancer alkaloid and DNA intercalator (Vavreckova & Ulrichova, 1994), while chelerythrine, berberine and chelidonine have a smaller anticancer activity in vitro (Biswas, 2013) and in vivo (European Medicines Agency, 2011). Chelidonine induces apoptosis of cancer cells by inhibition of tubulin polymerization, telomerase of tumor cells and mitosis (Biswas, 2013, Gausage et al., 2001). For the variety of alkaloids binding with the DNA molecule is responsible alkaloid structure of Chelidonium majus (Bashmakova, et al. 2008). During transport the blood alkaloids weakly bound to albumin in a quaternary structure, while alkaloids are covalently bonded in the tertiary structure with other proteins and bacteria. The binding of alkaloids in the tertiary structure is responsible for the antibacterial effect of alkaloids (Malikova et al., 2006).

## CONCLUSION

The results of this study have shown higher antiproliferative activity of the ether extract in comparing with the aqueous extract of haulms of Chelidonium majus on all of studied human tumor cell lines. Both haulms extracts of Chelidonium majus were the most effective on CEM cells. This study have pointed out the dual effect of the Chelidonium majus haulms extract, which is dependent on the concentration of used extract and the polarity of solvent (aqueous versus organic). In the aqueous extract of the haulms of Chelidonium majus are polar constituents (eg sanguinarine, chelerythrine, chelirubine and others), while in the ether solution are lipophilic compounds. The higher antiproliferative activity of ether haulms extract of Chelidonium majus is probably the result of higher content of active lipophilic substances (eg dihydrosanguinarine, dihydrochelerythrine, dihydrochelirubine and others), that easily cross the cell membrane and preferentially bind to the various proteins and change their biological activity (Motilal & Gopinatha, 2007). Both structures KBA and TBA can be present in different ratio. The ratio between the quaternary and tertiary form decides about the bioactivity of alkaloid. Benzo[c]phenanthridine alkaloid TBA pass through the cell membrane in tertiary lipophilic structure and inside the cell it is converted to quaternary hydrophilic structure of benzo[c]phenanthridine alkaloid (KBA), that has an antibacterial, antiviral and cytotoxic effect (Ishikawa, 2001). Alkaloids are reactive inside the cell. They can interact with with proteins, DNA and lipids (Ulrichova et al., 2001). Several studies confirmed the cytotoxic effect of Chelidonium majus in the treatment of cancer (Lanvers-Kaminsky et al., 2006).

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