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In Vitro Cytotoxic Activity of *Chelidonium majus* extract using Different Types of Cell Lines

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A B S T R A C T

Cancer is becoming a health problem in all over the world, searching for anticancer therapy from plants natural products has been one of the sustainers of cancer chemotherapy approximately for the past half a century. *Chelidonium majus* (Poppy Family) is a plant which is distributed in nature and has been used as a traditional medicine. The plant extract showed high content of alkaloids which revealed cytotoxic effect in a cell type and dose dependent way. Cytotoxic analysis of alkaloids extract from *Chelidonium majus* using MTT assay on different cell lines. 100 grams of the plant material powdered and extracted with methanol using Soxhlet installation, the extract was subjected farther more for acid-base extraction and purification with chloroform. Cytotoxic effect of total alkaloid was estimated using MTT-assay manufacturer's instructions. The total alkaloid extract of *C. majus* exhibited remarkably significant cytotoxic effect on (Hep-G2, MCF-7 and A-549) cell lines in a dose dependent and cell type dependent manner. The extract illustrated considerable cytotoxic activity against various types of cancer cell lines *in vitro.* The antitumor activity was extremely efficient in a dose dependent manner it displayed decreased viability of tumor cells.

Introduction

Chelidonium majus L. (poppy family), known as greater celandine, the plant grows wild in parts of Asia, North America, Southern and Central Europe (Colombo and Bosisio, 1996).

Due to their notable pharmacological effects, *Ch. majus* is widely used in traditional and modern medicine for the treatment of liver diseases, gastrointestinal tract, and there are also some data which demonstrated the use of this herb for the prevention and treatment of cancer and tumors (Venkatesh *et al.*, 2011; De Melo *et al.*, 2011). The plant contains, isoquinoline alkaloids as tertiary and quaternary benzo[c] phenanthridine alkaloids such as (sanguinarine, chelidonine, chelerythrine, berberine, protopine and coptisine), flavonoids, and phenolic acids (Colombo and Bosisio, 1996; Barreto *et al.*, 2003). Both crude extracts of *C. majus* and purified compounds derived from it exhibits a wide variety of biological activities that includes, anti-inflammatory (Lee *et al.*, 2007), antimicrobial

(Kokoska *et al.,* 2002), immunomodulatory (Song *et al.,* 2002), antitumor, choleretic, hepatoprotective, analgesic (Gilca *et al.,* 2010), which are in concordance with the traditional uses of *C. majus*.

Different alkaloids of *C. majus* have the following activities that might be responsible for its anticancer effect:

Reduced telomerase activity by chelidonine (Noureini and Wink, 2009),

Cancer cell death by apoptosis (Noureini and Wink, 2009; Habermehl *et al.,* 2006; Philchenkov *et al.,* 2008),

And blister cell death (Philchenkov *et al.,* 2008),

Arrest of mitosis by inhibition (Noureini and Wink, 2009).

A number of studies suggest that Ukrain TM (an anticancer drug whose major components are *C. majus* alkaloids chelidonine, sanguinarine, chelerythrine, protopine, and allocryptine) (Habermehl *et al.,* 2006) exerts multiple selective effects on cancer cells:

Cytotoxic effects on cancer cells without negative effects on normal cells (Hohenwarter *et al.,* 1992);

Radio-sensitizing effects on cancer cells, but radio-protective effects on normal cells (Cordes *et al.,* 2002).

Materials and Methods

Preparation of plant material and extract

The plant material extraction and purification followed (Bugatti *et al.,* 1991) and (Ramawat and Merollin, 2007) in brief: The plant material was collected from Botanic Planet a store of herbs and natural product in (Brampton, Ontario, Canada) as grinded dried parts (aerial part only). 100 grams of the plant material powdered by using grinder and packed loosely in thimble, the plant extracted with organic solvent 85% methanol (500ml) using a Soxhlet installation for 24hrs.

The extract then half evaporated and then combined in beaker. Using sulphuric acid the extract pH reduced to value (pH 2) (Checked by litmus paper), then extracted in a separatory funnel with 100 ml chloroform. Both phases (A for aqueous phase, B for non-polar phase) were collected separately.

The aqueous phase (A) basified by ammonium hydroxide until the pH reached to the value (pH 9) (Checked by litmus paper) and extracted again with organic solvent chloroform in separatory funnel, the two phases were collected separately (A+ for aqueous phase, B+ for non-polar phase). A+ phase was extracted again with chloroform till Dragendorff's test was negative. A, B and B^+ phases were concentrated by evaporation then dried and calculated for total Alkaloid. During extraction and purification steps (A, A^+, B and B^+ phases) samples were collected and estimated for alkaloid by using Dragendorff's and Mayer's test (Figure 4).

Cell culture

Hep-G2 (Human liver cancer cell line), MCF-7(Breast cancer cell line) and A-549 (Human alveolar adenocarcinomic cell line) were collected from National Center for Cell Science (NCCS Pune / India). The cells were maintained in RPMI-1640 medium which supplemented with 10% fetal bovine serum, 10ug/ml Ciprofloxacin all from (Sigma, India). Cells were incubated at 37°C in an atmosphere of 5% CO^2 and absolute humidity. Cell number and viability were determined using ethidium bromide.

Cytotoxicity study

MTT assay

Cytotoxic effect of total alkaloid was estimated using MTT-assay according to manufacturer's instructions (HiMedia, India) (http://himedialabs.com/TD/CCK003.pdf) in brief: The cell suspension seeded in a 96-well plate and at required cell density (20,000 cells per well), without the test agent and then incubated to grow and adhere for about 12 hours. After incubation the plate were taken out and examined under inverted microscope to ensure cells adherent, appropriate concentrations of the extracted total alkaloid were added (5,50,100,150 to 200 µg/mL) and then incubated for 24hrs at 37ºC in 5% CO2 atmosphere.

After the incubation period, 10 % of MTT reagent (5mg/ml) were added to a final concentration of total volume. The plates were wrapped with aluminum foil to avoid exposure to light and incubator and incubated for 3 hours.

During that time, metabolically active viable cells reduced yellow, MTT was reduced to purple formazan, due to activity of mitochondrial dehydrogenase. The MTT reagent was carefully removed and then 100 µl of solubilisation solution dimethylsulfoxide (DMSO) was added to each well. A gentle shake was done to enhance dissolution. Occasionally, pipetting up and down was required to completely dissolve the MTT formazan crystals especially in dense cultures. The absorbance were values read by ELISA reader at 570nm and 630nm which was used as reference wavelength The percentage of inhibition was calculated according to the following equation;

Inhibition % = 100- [(optical density of test wells/optical density of control wells)] × 100.

Experiment controls were medium control without cells, medium with cells but without total alkaloid (negative control) and medium with cells treated with berberine (positive control).

Statistical analysis

The Statistical Analysis System- SAS (2014) program was used to analyse effect of different concentration for studying parameters (viability %). Least significant difference –LSD test (ANOVA) was used to significantly compare between means in this study (SAS, 2014).

Results and Discussion

Hep-G2 human hepatocyte carcinoma

In Hep-G2 the percentage of viability was clarified in (Table 1) results were presented as mean ± SE. for non-treated cells the percentage was 100% at 24hrs of incubation. The percentage of viability started to decrease at concentration 150, 300, 450, 600 and 750 µg ml^{-1} to reach 77.67 ± 1.52, 51.34 ± 2.34, 5.73 ± 0.52, 1.17 ± 0.44, and 0.59 ± 0.17 % respectively at 24hrs of incubation. Cell proliferation was significantly decreased following treatment with the *C. majus* extract (Figure 1) in a concentration dependent manner (Figure 5) ($P<0.01$). The IC50 was observed at 282.86µg/ml after 24hrs of treatment.

MCF-7 human breast cancer

The viability of MCF-7 clarified in (Table 2) results were presented as mean + SE. for non-treated cells the percentage was 100% at 24hrs of incubation. The percentage of viability started to decrease at concentration 150, 300, 450, 600 and 750 µg ml-1 to reach 84.12 ± 3.45, 76.04 ± 3.22, 64.55 ± 5.09, 50.21 ± 2.66, and 29.92 ± 0.86 % at 24hrs of incubation**.**

Table.1 Effect of concentration in viability // Cell line: Hep-G2

Concentration (µg/ml)	Mean ± SE of Viability (%)
Control	100.00 ± 0.00 a
150	77.67 ± 1.52 b
300	51.34 ± 2.34 c
450	5.73 ± 0.52 d
600	1.17 ± 0.44 d
750	0.59 ± 0.17 d
LSD value	7.319 **
P-value	0.0001

** (P<0.01). Means having the different letters in same column differed significantly.

Table.2 Effect of concentration in viability // Cell line: MCF-7

Concentration (µg/ml)	Mean ± SE of Viability (%)
Control	100.00 ± 0.00 a
150	84.12 ± 3.45 b
300	76.04 ± 3.22 c
450	64.55 ± 5.09 d
600	50.21 ± 2.66 e
750	29.92 ± 0.86 f
LSD value	6.882 **
P-value	0.0001

** (P<0.01). Means having the different letters in same column differed significantly.

Table.3 Effect of concentration in viability // Cell line: A-549

Concentration (µg/ml)	Mean ± SE of Viability (%)
Control	100.00 ± 0.00 a
150	53.09 ± 0.87 b
300	36.42 ± 3.11 c
450	3.06 ± 0.18 d
600	1.07 ± 0.09 d
750	0.85 ± 0.09 d
LSD value	7.924 **
P-value	0.0001

** (P<0.01). Means having the different letters in same column differed significantly

Fig.1 Cytotoxic effect of *C. majus* on Hep-G2 cell line

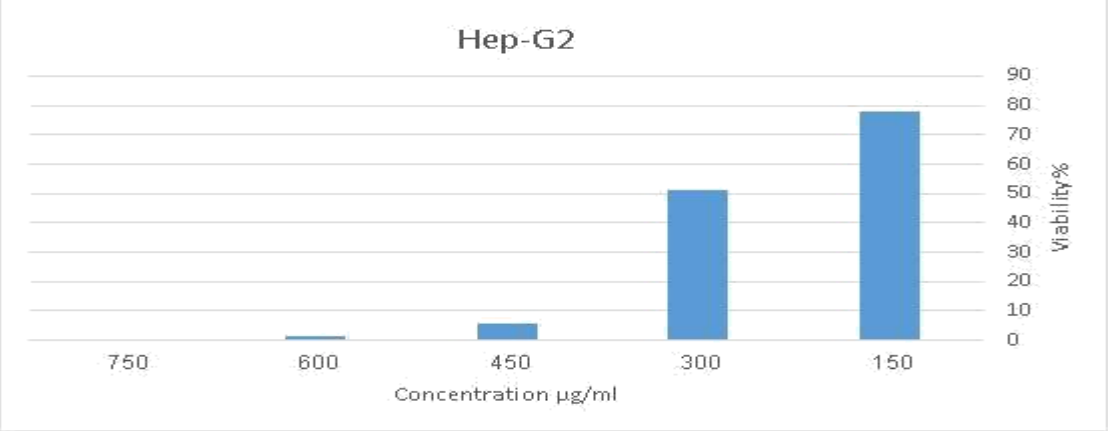


Fig.2 Cytotoxic effect *of C. majus* on MCF-7 cell line

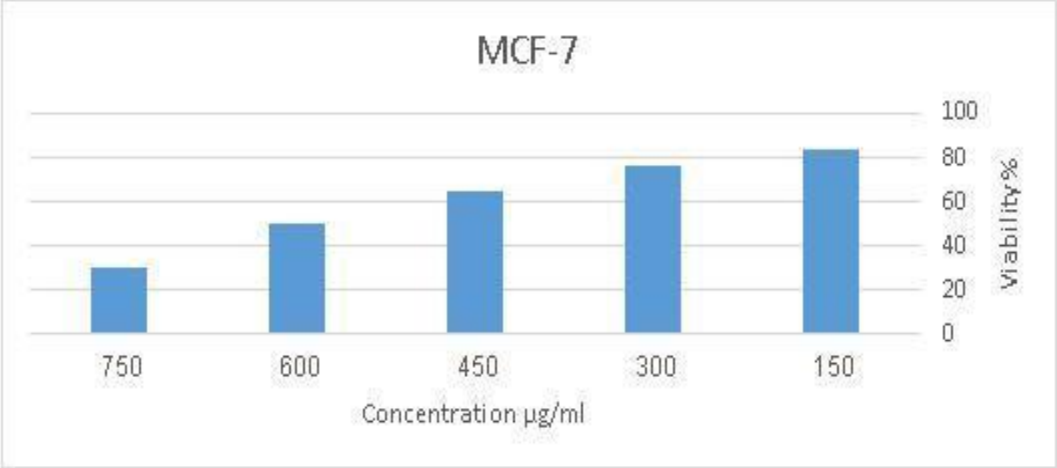


Fig.3 Cytotoxic effect of *C. majus* on A-549 cell line

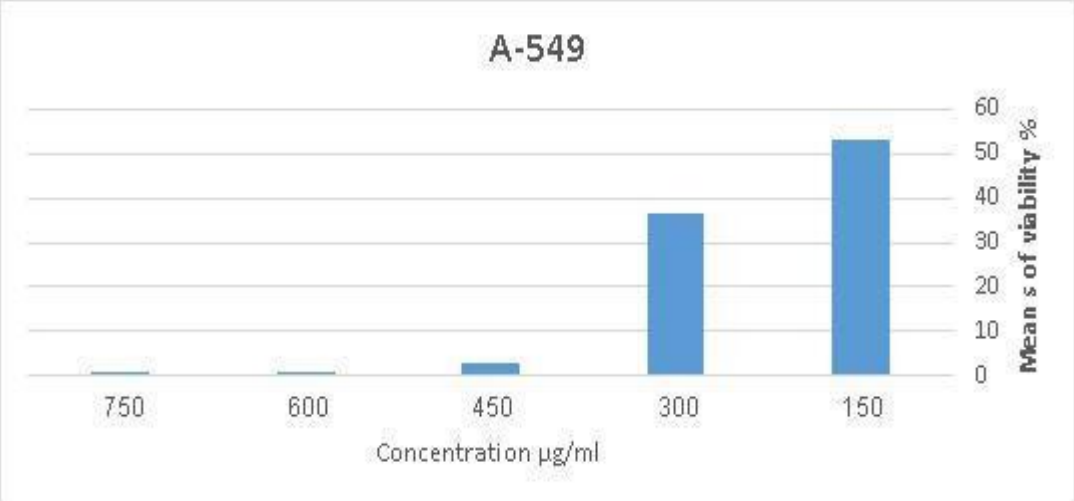


Fig.4 Estimation of total alkaloid by Dragendorff's (A) and Mayer's (B) reagent

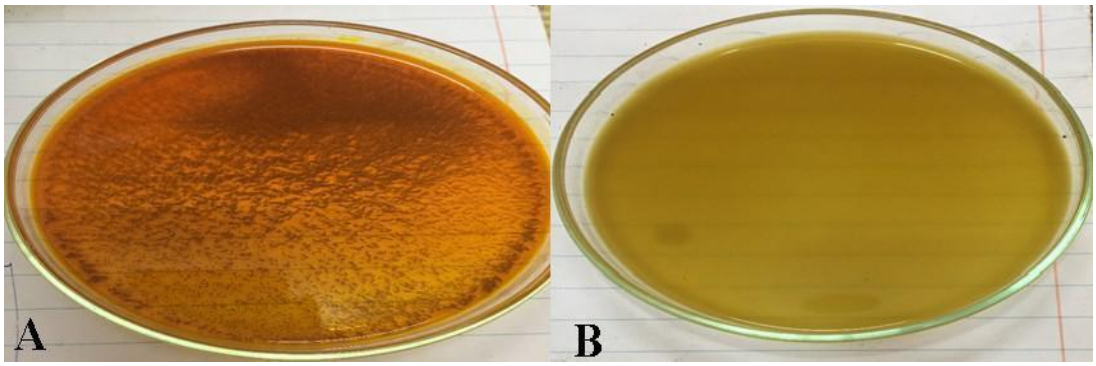


Fig.5 Inverted microscopy image of Hep-G2 treated and untreated (control) cell lines respectively. Morphology was visualized and photographed under light microscope (magnification, x100)

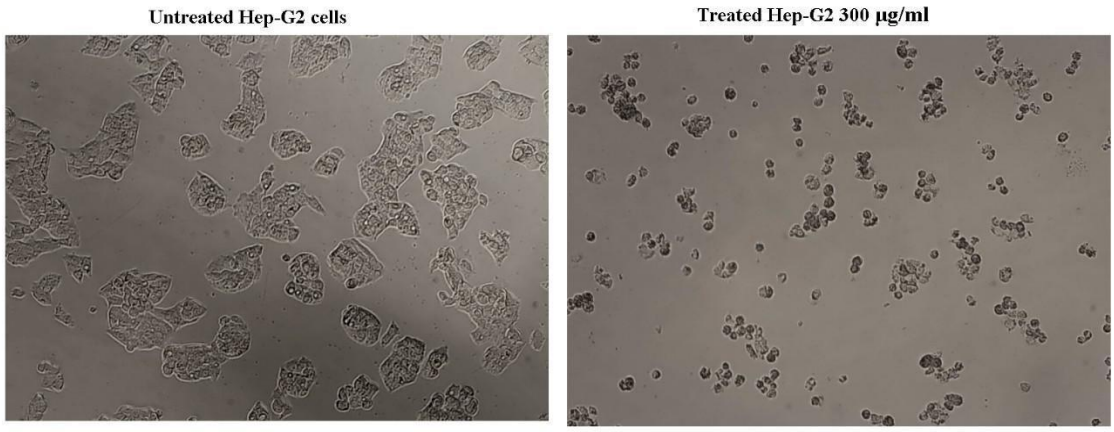


Fig.6 Inverted microscopy image of MCF-7 treated and untreated (control) cell lines respectively. Morphology was visualized and photographed under light microscope (magnification, x100)

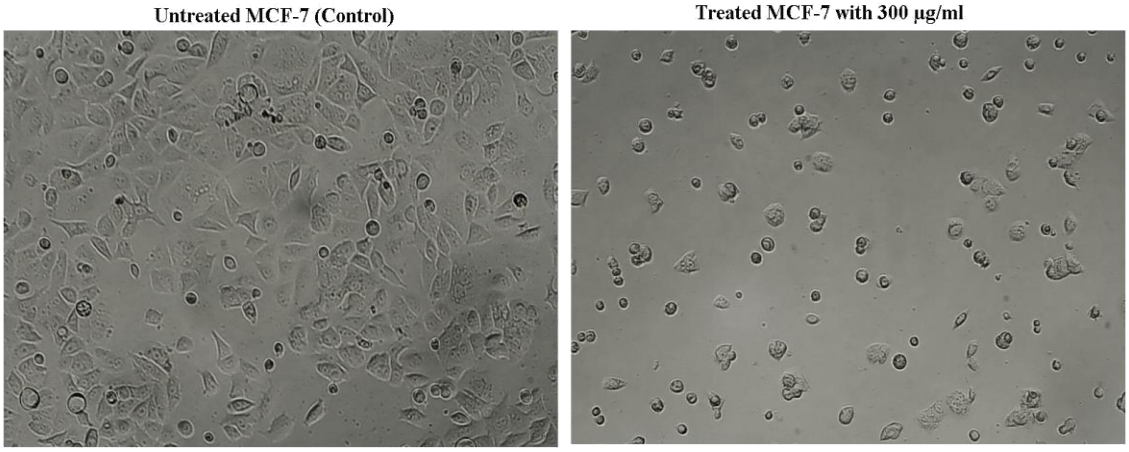
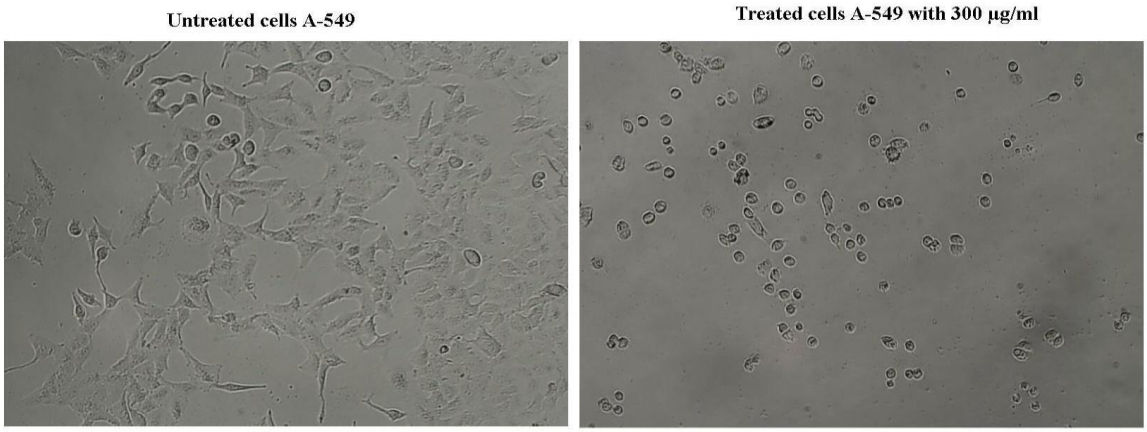


Fig.7 Inverted microscopy image of Hep-G2 treated and untreated (control) cell lines respectively. Morphology was visualized and photographed under light microscope (magnification, x100)



Cell proliferation was significantly decreased following treatment with the *C. majus* extract (Figure 2) in a concentration dependent manner (Figure 6) (P<0.01). The IC50 was observed at 570.879 µg/ml after 24hrs of treatment.

A-549 Human Lung Carcinoma

The percentage of growth inhibition for A-549 was clarified in (Table 3) results are presented as mean ± SE. for non-treated cells the percentage was 100% at 24hrs of incubation. The percentage of viability started to decrease at concentration 150,300,450,600, and 750 µg ml-1 to reach 53.09± 0.87, 36.42 ± 3.11, 3.06 ± 0.18, 1.07 ± 0.09, and 0.85 ± 0.09 % at 24hrs of incubation.

Cell proliferation was notably decreased following treatment with the *C. majus* (Figure 3) extract in a concentration dependent manner (Figure 7) (P<0.01). The IC50 was observed at 172.12µg/ml after 24hrs of treatment.

In association with other studies done by (ZareShahneh *et al.*, 2013) which showed the cytotoxic effect of the crude methanolic extract of *Chelidonium majus* were probed in vitro using MTT assay. MTT results surfaced that cytotoxic effect alongside Non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), human acute myelocytic leukaemia (KG-1A), human breast carcinoma (MCF-7 cells), and human Prostate Cancer (PC3) cell lines in a dose-dependent manner. Milena Deljanin *et al.*, (2016) demonstrated that *C. majus* extract decreased viability of tumor cells. Treatment with C. majus extract culminated in time- and dose-dependent proliferation in cytotoxicity. The cytotoxic effect of the extract on MCF-7 asserted IC50 value which was 179,35µg/ml.

In this paper the results showcased cytotoxic effect of extracted alkaloid on different type of cell line. Where it appeared that toxicity was in a dose dependent manner as well as type dependent manner, which might be due to cell type and sensitivity to the extract.

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Conflict of interest

Authors declare no conflict of interest.

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