

**Original Article****Investigation of *in vitro* Cytotoxicity of *Chelidonium majus* against *Leishmania Major***

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**Abstract**

One of the public health issues in the endemic areas, especially in the Middle East region would be the Leishmaniasis. The suggested cure for leishmaniasis is pentavalent antimonials. These medications have drastic side effects and the risk of relapse. On the other hand, nowadays use of herbal remedies as safe and cost-effective treatments have been increased. Therefore this study was designed to determine *in vitro* anti-leishmanial activity of methanol extracts of greater celandine (*Chelidonium majus*) against *Leishmania major*. Greater celandine extract was added to *L. major* promastigotes and intra-macrophagic amastigotes. After 24, 48 and 72 h *in vitro* culture the percentage of promastigotes viability was calculated by direct counting method and MTT assay. Cytotoxicity in intra-macrophagic amastigotes was evaluated by direct counting method. Viability in minimum dose and maximum dose-treated groups (1.5 and 90 µg/ml) after 24 h, was 55.52% and 36.34%, respectively. After 48 h, it was 40% and 25.26% and after 72 h, it was 62.18% and 38.45%, respectively. The half maximal inhibitory concentration (IC<sub>50</sub>) was 0.92 µg/ml, after 24 h. Cytotoxicity in intra-macrophagic amastigotes treated by 3 µg/ml dose after 24 and 48 h, was 33.23% and 50.34%, respectively. It could be concluded that greater celandine methanolic extract has *in vitro* cytotoxic effect on the *L. major* in time and dose-dependent pattern.

**Keywords:** amastigotes, celandine, *Chelidonium*, *Leishmania major*, promastigotes

**1. Introduction**

Cutaneous, mucocutaneous and visceral leishmaniasis are the main three forms of this parasitic disease (1). Cutaneous leishmaniasis is one of the most serious parasitic diseases which is endemic in the Middle East

region, particularly in Iraq. *Leishmania* is flagellate protozoa and causative agent of leishmaniasis (2, 3). Pentavalent antimonial compounds have been considered the first-line therapy for leishmaniasis. Several serious side effects have been listed for

pentavalent antimonial drugs such as: cardiotoxicity, reversible renal failure, pancreatitis, anemia, leukopenia, rash, headache, abdominal pain, nausea, vomiting, arthralgia, myalgia, thrombocytopenia, and transaminase elevation (4). The use of herbal remedies as safe and cost-effective treatments have been increased (5). One of the most important herbs in Chinese traditional medicine is named Greater celandine plant (*Chelidonium majus*) which belongs to the Papaveraceae family. This herb is spread around the world. Due to the antibacterial (against *Staphylococcus aureus*) (6), antifungal (7), and cytotoxic effects (8) of its methanolic extract, it has been used to treat several human disease such as warts, eczema, tumors, and skin diseases (9). In different parts of Greater celandine, root and aerial part, several alkaloids have been identified as follows: protoberberines, allocryptopine, protopine, sanguinarine, chelerythrine, and chelidonine (10). These alkaloids from *Chelidonium majus* have a significant inhibitory effect on mitochondrial respiration (11).

Therefore, this study was designed to identify *in vitro* anti-leishmanial activity of methanolic extract of greater celandine (*Chelidonium majus*) against *Leishmania major* amastigotes and promastigotes.

## 2. Materials and Methods

### 2.1. Methanolic Extraction

Greater celandine was purchased from herbal pharmacy. It was identified in our University with herbarium code 18740. Percolation method was used for plant extraction (using 80% methanol). Then, methanol was removed by rotary evaporator (IKE WERKE, RV06-ML) at 40 °C and low pressure and extract was concentrated. Following drying the extract it was packed and stored at 0 °C in a dark packing to prevent illumination. To prepare the stock solution the plant powder extract was dissolved in normal saline (2 mg/ml) and kept at -20 °C until use.

### 2.2. Study Design and MMT Assay

To evaluate the effect of plant on the *Leishmania* promastigotes: 10<sup>6</sup> cell/ml of *L. major* promastigotes in logarithmic phase was added to 96-well plate, then

greater celandine extract with 1.5, 3, 15, 30, 90 µg/ml concentrations was added to wells. The wells contained the promastigotes without plant extract, were considered as control group. Plates were incubated at 21 °C. Viability percentage of promastigotes was calculated by direct counting method and MTT assay after 24, 48 and 72 hours. A colorimetric method for evaluation of cell activity and viability is called MTT [3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay. In the current study the MTT assay was done based on the method previously described by Sylvester (12).

### 2.3. *L. major* Promastigotes

The promastigotes were cultured in the RPMI-1640 medium containing 10% heat-inactivated Fetal Bovine Serum (FBS), and was incubated at 21 °C.

### 2.4. Culture of Amastigotes

Macrophages was extracted from peritoneal cavity of laboratory mouse by injection and aspiration of 3 mL sterile PBS. Macrophages were cultured in 12-well plates. Before culturing, round sterile coverslips were placed in the wells, then 10<sup>5</sup> macrophages/mL added to wells with RPMI 1640 medium supplied with 10% FBS and 0.5% gentamicin. Plates were incubated at 37 °C supplied with 5% CO<sub>2</sub>. After 24 hours, 10<sup>6</sup> promastigote/ml located in statistic phase was added to wells, then plate was incubated at 37 °C and 5% CO<sub>2</sub> again. After 24 hours, the wells was washed by RPMI 1640 to remove additional promastigotes and dead cells, then fresh medium with 10% FBS was added to wells.

### 2.5. Antileishmanial Activity of Plant Extract against Amastigotes

Greater celandine extract (3 and 90 µg/ml concentrations) was added to wells, then plate was incubated at 37 °C and 5% CO<sub>2</sub> again. The well containing infected macrophages, without plant extract, was considered as a control group. In order to evaluate cytotoxic effect of plant extract on the amastigotes, cover slips were removed from the wells, fixed by absolute methanol and stained with Giemsa. Then, the number of amastigotes inside each macrophage was counted in 100 infected macrophages.

## 2.6. Statistical Analysis

Statistical analysis was done by SPSS software version 22, one-way analysis of variance (ANOVA) was used to data analysis. The percentage of killed amastigotes in treated groups was obtained compare to control group.

## 3. Results

The recorded data showed that the promastigotes viability treated by the highest and the lowest concentrations of greater celandine extract (1.5 and 90 µg/ml) after 24 h was: 55.52% and 33.73%, respectively. While the results showed that the promastigotes viability treated by the highest and the lowest concentrations of greater celandine extract (1.5 and 90 µg/ml) after 48 h, was: 40% and 25.26%, respectively. However, after 72 h, the promastigotes viability was: 62.18% and 38.45%, respectively. The recorded data showed that the half maximal inhibitory concentration (IC<sub>50</sub>) of greater celandine was 0.92 µg/ml, following 24 h.

The recorded data showed that the mean number of amastigotes in the control group following 24 and 48 h, was 12.64 and 15.86 cell/macrophage, respectively. While the results revealed that 9 and 8.76 cell/macrophage was identified in treated group by 3 µg/ml of plant extract and 7.5 and 6.45 cell/macrophage in treated group by 90 µg/ml of *Chelidonium majus* extract. The cytotoxicity percent in amastigotes was calculated compare to the control group. The cytotoxicity assay results showed that the cytotoxicity percentage was 33.23 and 40.64% in treated group with 3 µg/ml of plant extract, after 24 and 48 hours, respectively. In treated group with 90 µg/ml of plant extract, the cytotoxicity was 50.34% and 59.33%, respectively.

## 4. Discussion

The findings of the current study revealed that the greater celandine extract has time- and dose-dependent *in vitro* anti-leishmanial activity against the promastigotes and amastigotes of *L. major*. The highest

*in vitro* cytotoxicity belongs to 31 µg/ml at 48 h. It has been observed that viability increased at 72 h due to two reasons: firstly, using promastigotes in logarithmic growth phase and secondly, decrease of cytotoxic effect of *Chelidonium majus* extract. Cytotoxic effect of *Chelidonium majus* on the amastigotes was investigated just at 24 and 48 h, because most infected macrophages were destroyed at 72 h due to amastigotes proliferation. Also, two dose of plant extract was used to evaluate cytotoxic effect of *Chelidonium majus* on the amastigotes; one of them close to IC<sub>50</sub> (3 µg/ml) and another was the highest concentration (90 µg/ml). The highest cytotoxicity percentage in amastigotes was 59.34 and belonged to group treated with 90 µg/ml of plant extract at 48 h.

The current study was the first report of *in vitro* cytotoxic effect of *Chelidonium majus* on the Leishmania. Various alkaloids of *Chelidonium majus* reported to inhibit the growth of *Trichomonas vaginalis in vitro* (13). Yao, Zhou (14) showed that *Chelidonium majus* extract has anti-helminthic effect on the *Dactylogyrus intermedius* at a concentration of 0.9 mg/ml, with EC<sub>50</sub> value of 0.48 mg/ml after 48 h of exposure.

Previously published researches have shown that *Chelidonium majus* extract has antibacterial effects on many Gram-negative and Gram-positive bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Streptococcus mutans*, as well as fungi like *Candida albicans* and *Fusarium* (7, 15, 16).

## Authors' Contribution

Study concept and design: H. D. S.

Acquisition of data: K. M. H. and N. M. H.

Analysis and interpretation of data: A. M. R.

Drafting of the manuscript: W. K. A. and L. B. A.

Critical revision of the manuscript for important intellectual content: E. S. A., H. A. H. and F. K. H. A.

Statistical analysis: N. H. A.

Administrative, technical, and material support: K. M. H.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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