

## Effects of coadministration of artemisinin and iron on histopathological alterations in the AGS gastric adenocarcinoma cell line

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**Background/aim:** The aim of the present study was to examine the anticancer effects of combination treatment with artemisinin and iron on the human gastric cancer cell line.

**Materials and methods:** The AGS cell line was cultivated separately as a monolayer in culture flasks and different doses of 99% pure artemisinin with invariable doses of iron sulfate were added to the culture media and adhesive cells on the flask's bottom were stained with hematoxylin–eosin for a pathological assay.

**Results:** Damage to cancerous cells increased dose dependently and it was higher in the combination treatment groups (artemisinin plus iron). The histopathological changes were observed specially in the groups with high artemisinin concentration as cell swelling, nucleus swelling, and formation of small and large vacuolization. Necrotic changes as nucleus pyknosis were seen too. Changes in groups receiving both artemisinin and iron gradually became more severe with dose increase.

**Conclusion:** Pathologic studies showed that the cytotoxic effects of artemisinin were dose dependent and the presence of iron enhanced the artemisinin's anticancer potency.

**Key words:** Artemisinin, iron, AGS cell line, gastric cancer, apoptosis

### 1. Introduction

The anticancer effects of artemisinin and different combination therapies with it have been shown in many in vivo and ex vivo experiments such as in leukemia and breast and prostate cancer (1–8). Artemisinin, a traditional Chinese medicine, is derived from (the Chinese plant qinghao) *Artemisia annua*. Recently it has been widely used as an antimalarial and anticancer agent. This sesquiterpene phytolactone compound possesses an endoperoxide bridge that reacts with free iron and generates organic free radicals that cause induction peroxidation of the cell membrane's lipid (9–11). This process initiates apoptosis and necrosis of the cell and leads to cell death (7,12,13).

Gastric cancer is a major cause of mortality. Its prevalence is decreasing in industrial countries, but it is still the fifth global mortality cause after lung, breast, colorectal, and prostate cancer and it is third placed after lung and liver cancer in East Asia. Various kinds of gastric cancer exist based on the tissue involved. Adenocarcinoma is one of the most prevalent types of gastric cancer and it has been involved in 95% of reported mortalities (14,15).

The AGS cell line, a human gastric adenocarcinoma, is a suitable model for studying stomach cancer and evaluating cancer cell apoptosis (14).

The aim of our research was to study the anticancer effects of combination treatment with artemisinin and iron on the human gastric cancer cell line.

### 2. Materials and methods

The AGS (ATCC CRL1739) human stomach adenocarcinoma epithelial cell line was obtained from the National Cell Bank of Iran (Pasteur Institute, Iran). There were Roswell Park Memorial Institute (RPMI 1640) without antibiotics, with 10% fetal bovine serum (FBS), and nonessential amino acids in the cell media.

This line was cultivated separately as a monolayer in T25 culture flasks containing RPMI 1640 medium supplemented with 10% FBS. The cells were grown in an incubator at 37 °C with 5% CO<sub>2</sub> and 95% humidity. They were classified into 9 groups. Culture media refreshment was done every 3 days.

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Different doses of 99% pure artemisinin, with invariable doses of iron sulfate (10 µg/mL) were added to the culture media. Nine different groups were classified. Four groups received one dose of artemisinin (0.15, 0.3, 0.6, and 1.2 µg/mL) with iron sulfate, and the same doses of artemisinin were added to another four groups (3) without iron. One group was considered the iron control (Table).

The cellular alterations were checked and photographed under an inverted microscope every 12 h. At the end of the study, adhesive cells on the flask's bottom were fixed with methanol and stained with hematoxylin–eosin for a pathological assay.

There were three culture media for each dose during this experiment in order to confirm the accuracy of the histopathological results.

### 3. Results

Damage to cancerous cells increased dose dependently and was higher in the combination treatment groups (artemisinin plus iron). In the histopathological investigations, the changes observed were as follows. In the artemisinin groups (without iron) pathological changes were observed only in two groups with artemisinin concentration of 0.6 and 1.2 in the form of cell swelling, formation of small vacuoles in small quantities in cells, and swelling of the cell nucleus.

Changes in groups receiving both artemisinin and iron gradually became more severe with dose increase. Some cells showed mild swelling with a dose of 0.15. In a dose of 0.3, cell and nucleus swelling were seen. In doses of 0.6 and 1.2, severe cell swelling and severe and large vacuolization of cells were seen. Some cells showed necrotic changes in the form of nucleus pyknosis. Fragmentation of cell cytoplasm was also observed. Dislodging of the cells from

the bottom of the culture medium and depletion of the bottom were minimal in the group of 0.6 but maximal in the group of 1.2.

These lesions were severely dependent on dose and time and became more severe over time. However, in the last investigation at 60 h some new mitosis was observed at the bottom of the culture medium in compensation for depleted cells. In the group of 1.2 with iron, in the last hours necrotic lumps sticking together were observed (Figures 1–3).

### 4. Discussion

Because of the metabolic activity of cancerous cells, they have massive quantities of iron deposits; on the other hand, artemisinin has selective uptake through transferrin receptors (related to iron) and these factors lead to the selective cytotoxicity of artemisinin on neoplastic cells. However, there is no effect on normal cells (selective toxicity) (16).

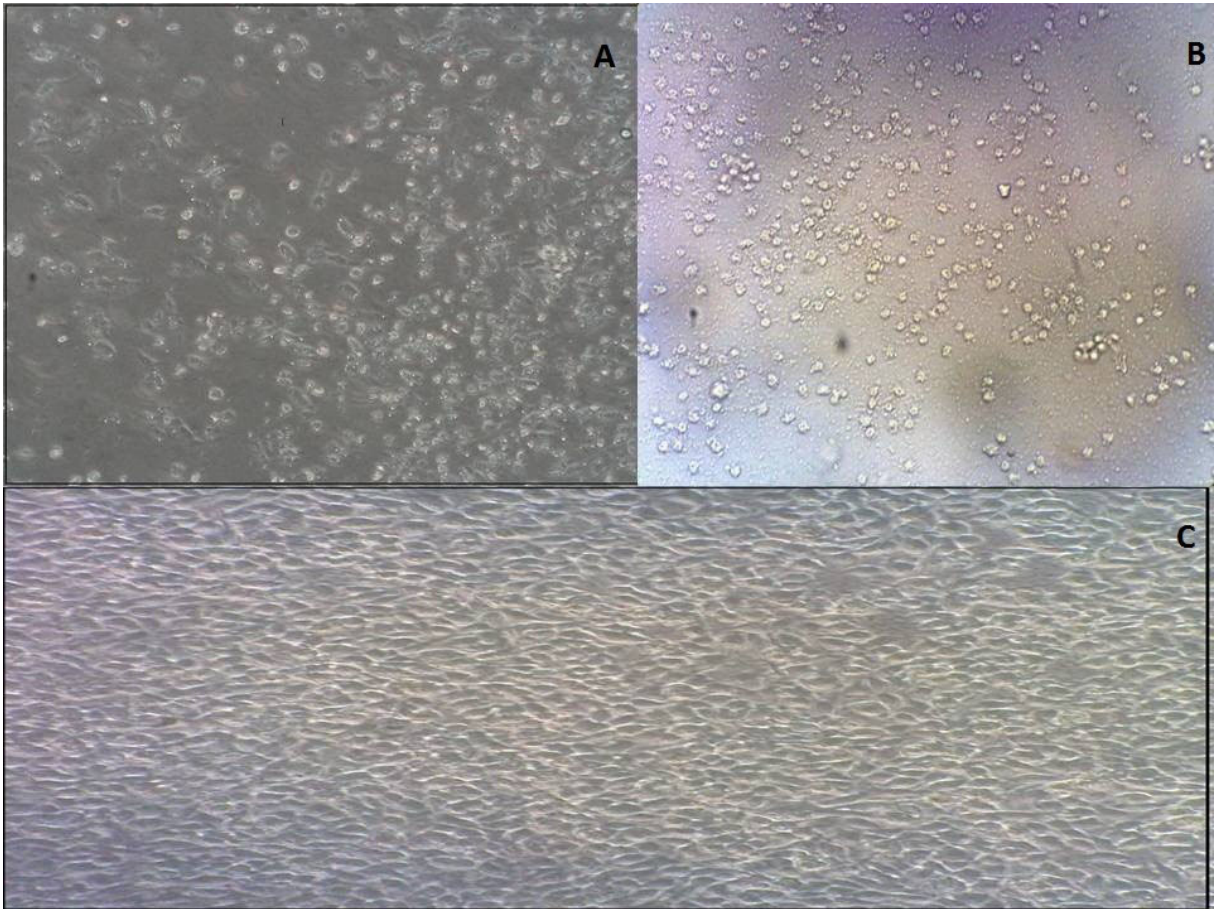
In the present study, the anticancerous effects of different doses of artemisinin alone and mixed with ferrous sulfate were studied.

Lai et al. (17) showed that iron and artemisinin cause formation of free radicals and were highly toxic for leukemic and breast cancer cells.

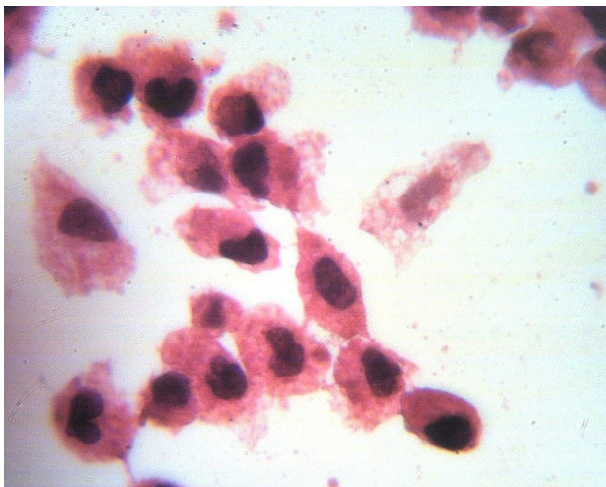
Efferth et al. (12) stated that iron and transferrin increased the cytotoxicity of artemisinin toward leukemia and astrocytoma cells. This enhancement was 1.5–10.3-fold compared with artemisinin alone. In addition, Shahbazfar et al. (3) found that combination therapy with artemisinin was more effective than single therapy against cancerous cells of bladder and breast cancer. Furthermore, the synergistic effects of iron and artemisinin have been indicated in other studies.

**Table.** Drug composition of different doses of artemisinin with invariable dose of iron sulfate (10 µg/mL) in the treatment groups.

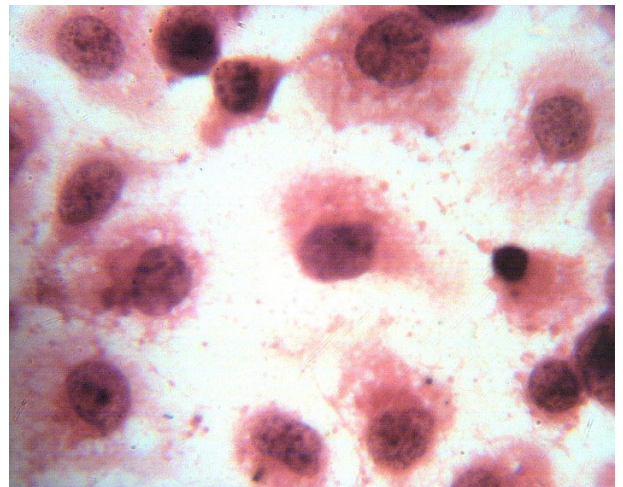
Group	Agents
1	Artemisinin (0.15 µg/mL) + iron sulfate
2	Artemisinin (0.3 µg/mL) + iron sulfate
3	Artemisinin (0.6 µg/mL) + iron sulfate
4	Artemisinin (1.2 µg/mL) + iron sulfate
5	Artemisinin (0.15 µg/mL)
6	Artemisinin (0.3 µg/mL)
7	Artemisinin (0.6 µg/mL)
8	Artemisinin (1.2 µg/mL)
9	Iron control



**Figure 1.** (A) Cell swelling and dislodging of the cells from the bottom of the culture medium in group 3 (artemisinin 0.6 µg/mL + iron sulfate); invert microscopy; hour 60; AGS cell line ×40; (B): Cell swelling, dislodging of the cells from the bottom of the culture medium, necrotic lumps sticking together in group 4 (artemisinin 1.2 µg/mL + iron sulfate); invert microscopy; hour 60; AGS cell line ×40; (C): Cells were steady in group 6 (artemisinin 0.3 µg alone) and did not show any specific pathologic changes; invert microscopy; hour 60; AGS cell line ×40.



**Figure 2.** Fine vacuolation in cytoplasm of cells in group 7 (artemisinin 0.6 µg alone); H and E staining; hour 60; AGS cell line ×1000.



**Figure 3.** Several areas of fine vacuolation in the cytoplasm of cells in group 8 (artemisinin 1.2 µg alone). One necrotic cell with pyknotic nucleus is shown; H and E staining; hour 60; AGS cell line ×1000.

In conclusion, artemisinin has been studied in several cancer cell lines but there were no data about artemisinin/iron combination treatment against the AGS cell line according to our search. The results of our study showed that the addition of iron enhanced the cytopathic effects of artemisinin against AGS cancer cell lines.

Pathologic studies showed that the cytotoxic effects of artemisinin were dose-dependent and the presence of iron enhanced artemisinin's anticancer potency.

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