

## Investigation of The Antiproliferative Effect of Colchicine on SNU-1 Gastric Cancer Cells

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### Research Article

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### ABSTRACT

In this study, colchicine's cytotoxic effects on SNU-1 cells were examined, and a probable mechanism behind its cytotoxicity was revealed. According to the results of the study, colchicine displayed considerable cytotoxicity with an IC<sub>50</sub> value of 14.81ng/ml when it was administered to the cells for 24 hours at different doses ranging from 5 to 100ng/ml. Furthermore, according to mechanistic studies, usege of colchicine significantly increased both early and late apoptotic cells in flow cytometry experiments. The late apoptotic cell population percentage in the control group (5.14 ± 1.27%) dramatically increased to 22.83 ± 1.38% in 14.81ng/ml colchicine treated cells. The early apoptotic cell population percentage in the control group (2.00 ± 1.12%) increased to 6.57 ± 2.35% in 14.81ng/ml colchicine treated cells. ELISA method was used to evaluate how colchicine affects the expression of pro- and anti-apoptotic proteins in SNU-1 cells. Colchicine treatment increased pro-apoptotic Bax and cleaved caspase 3 activities, while anti-apoptotic BCL-2 levels decreased. It is concluded that colchicine increases apoptosis in SNU-1 cells, which leads to an overall increase in cell death. Colchicine's promise as an anticancer drug to treat stomach cancer, however, needs additional research to be determined.

**Keywords:** Gastric cancer, Colchicine, Antiproliferative effect, Apoptosis.

## Introduction

Gastric cancer(GC) is a multifactorial disease, where many factors can influence its development, both environmental and genetic[1]. Globally, GC, the most prevalent form of gastrointestinal cancer, is the leading cause of death[2]. Every year, around 990,000 people are diagnosed with GC worldwide, of whom approximately 738,000 die[3]. GC is the fourth most common incident cancer and the second most common cause of cancer death[4]. The survival rate for advanced GC is less than 12 months[5]. GC is still a global health problem as a very aggressive malignancy with a complex nature[6]. Therefore, alternative prevention, considered as proper nutrition, early detection and follow-up of appropriate treatments, leads to a reduction in recorded cases[7]. There are still treatment options available for those with GC, such as surgery, chemotherapy, and radiotherapy[8]. Nonetheless, the most effective treatment for patients with the incurable condition is chemotherapy, preventing tumor invasion and metastasis [9]. Luo et al. (2010) implies that its effects are stable with overall 5-year survival ratios ranging from 5% to 15%[10]. Despite recent advancements in treatment of GC, overall survival statistics for patients with gastric cancer continue to be poor, which implies the urgency of discovering and developing new therapeutic approaches or agents for GC. According to Bhat and Singh (2008), a growing number of naturally occurring bioactive compounds have been investigated as potential anticancer medications

due to their high effect power and low damaging effect, in view of their ability to affect key stages in the genesis of cancer[11]. Because the rise in mitosis, one of the most well-known characteristics of cancer cells, depends on the arrangement microtubules, agents that target microtubules are being investigated as potential targets for chemotherapy[12]. In addition, studies by Huang et al. (2015) states that that a variety of plant-derived anti-microtubule medications, including vincristine, vinblastine and paclitaxel, have begun to be involved in clinical practice as chemotherapeutics or under laboratory work to gain approval for chemotherapy[13]. One of plant-derived anti-microtubule medications, called colchicine, is an alkaloid found in meadow saffron (*Colchicum autumnale* L.) and has been used for thousands of years to treat acute gout attacks and other inflammatory disorders[14]. According to the study by Kaplan et al. (1986), colchicine has been shown to be effective against some types of cancer and some liver diseases [15]. Colchicine exerts reduces an antiproliferative impact by hindering microtubule formation, resulting in the mitotic arrest and cell death through apoptosis [16]. Due to their effectiveness in inhibiting mitosis, colchicine and its semi-synthetic derivatives have the potential to be employed as chemotherapeutic drugs for the treatment of cancer [17]. Additionally, cancer cells have a much higher

mitotic rate and are more sensitive to colchicine than normal cells[18].

Colchicine has been proven in numerous studies to have antiproliferative effects on a variety of cancer cell lines, but the underlying processes are still unknown. The aim of the current study was to investigate the antiproliferative effects of colchicine on gastric carcinoma cells and the mechanisms behind colchicine's antiproliferative effects on gastric cancer cells.

## Material and Methods

### Cell Line and Cell Culture

American Type Culture Collection provided the SNU-1(CRL-5971) cell line (ATCC, USA) for the study. For SNU-1 cultures, RPMI-1640 media (Sigma-Aldrich) was employed. 10% Fetal Bovine Serum (FBS) (Gibco, Thermo Fisher Scientific), antibiotic combinations of 50 U/mL penicillin/streptomycin (Gibco, Thermo Fisher Scientific) was added to this medium. Cells were cultured at 37°C in a humidified environment with 5% CO<sub>2</sub>. Before treatment, DMSO-dissolved colchicine (Sigma-Aldrich) was diluted in culture media to have a final DMSO level of less than 0.1%.

### Cell Viability Assay

XTT assay (Roche Diagnostic, Germany) was employed to assess the antiproliferative activity of colchicine against cancer. Briefly stated, the cells were incubated in 96-well cell culture plates with an adjusted density of  $1 \times 10^4$  cells per well in triplicates for 24 hours with chosen colchicine concentrations (5, 10, 25, 50, 100ng/ml). A 50- $\mu$ L XTT labelling cocktail was then added to each well to measure living cells, and the cells were then incubated for an additional 4 h. The absorbance was then measured at 450 nm using an ELISA microplate reader (Epoch, Biotec, USA). The cell viability was recorded as % related to control (% of control). Graph Prism 7 software (GraphPad, Software, Inc., USA) was used to determine the half maximal inhibitory concentration(IC<sub>50</sub>) values of colchicine in examined cell line, and the IC<sub>50</sub> value of colchicine in SNU-1 gastric carcinoma cells was used in the ELISA and flow cytometry experiments that are detailed below.

### Annexin V Binding Assay

Prepared SNU-1 gastric carcinoma cells were initially put in six-well plate. The measured IC<sub>50</sub> concentration of colchicine was applied to the cells. Cells were harvested after 24 hours of incubation and then incubated with Muse™ Annexin V & Dead Cell kit reagent. The method employed during the procedure was the one advised by the manufacturer. Four distinct populations—live, early apoptotic, late apoptotic, and dead—were observed on the Cell Analyzer (Muse, Millipore) using Annexin V and/or 7-AAD positive.

### Bax, Cleaved Caspase 3 and BCL-2 Expression Analyses

Bax ELISA Kit (Abcam, Catalog #ab199080), Caspase 3 (cleaved) ELISA Kit (Invitrogen, Catalog #KHO1091), BCL-2

ELISA Kit (Invitrogen, Catalog #BMS244-3) were used, respectively, to measure the levels of Bax, cleaved caspase 3, BCL-2 proteins in the colchicine-treated and untreated SNU-1 gastric carcinoma cells. Briefly, the IC<sub>50</sub> concentration of colchicine as determined by antiproliferative effect experiments was applied to SNU-1 cells planted in a 6-well plate for 24 hours. After that, Bax, BCL-2, cleaved caspase 3 levels in the cell lysates were assessed in accordance with the manufacturer's instructions after colchicine-treated and untreated SNU-1 gastric carcinoma cells were lysed using the lysis buffer. Using the BCA assay, the total protein concentration in SNU-1 cells were also determined (Pierce Biotechnology, Rockford, IL, USA).

### Statistical Analysis

Each experiment was performed in triplicate, and the findings were provided as mean standard deviation. The post hoc Dunn test, Mann-Whitney test, and Kruskal-Wallis ANOVA test were carried out when necessary to compare the variables measured as a result of the treatment with colchicine with the control. A statistical difference was considered significant when  $P < 0.05$ .

## Results

### Colchicine Suppressed the Proliferation of Snu-1 Gastric Carcinoma Cells

Initially, colchicine's antiproliferative effect was determined in SNU-1 gastric carcinoma cells. Colchicine significantly reduced the growth of the SNU-1 cells at concentrations of 10ng/mL and higher when compared to untreated cells, as seen in Fig. 1 ( $P < 0.01$ ). Colchicine's IC<sub>50</sub> value was calculated to be 14.81 ng/mL for 24 hours in SNU-1 cells.

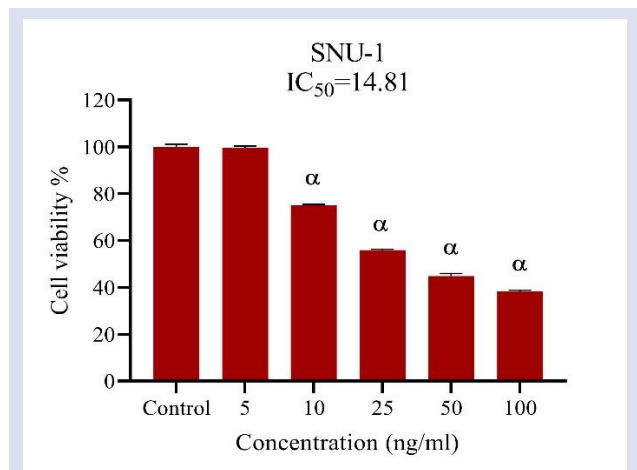


Fig. 1. Colchicine's effects on the viability of SNU-1 gastric carcinoma cells. Colchicine was applied to the cells for 24 hours at a concentration range between 5 and 100 ng/mL, and the number of viable cells was assessed using the XTT assay. The data are provided as a proportion of viable cells compared to the untreated control. The data are the mean and standard deviation from two separate experiments. The differences are  $^{\alpha}P < 0.01$  compared with control.

### Colchicine-induced Apoptosis of Snu-1 Gastric Carcinoma Cells

Initially, flow cytometric analysis and an Annexin V-FITC staining assay were conducted to evaluate the apoptotic effects of colchicine on SNU-1 cells.

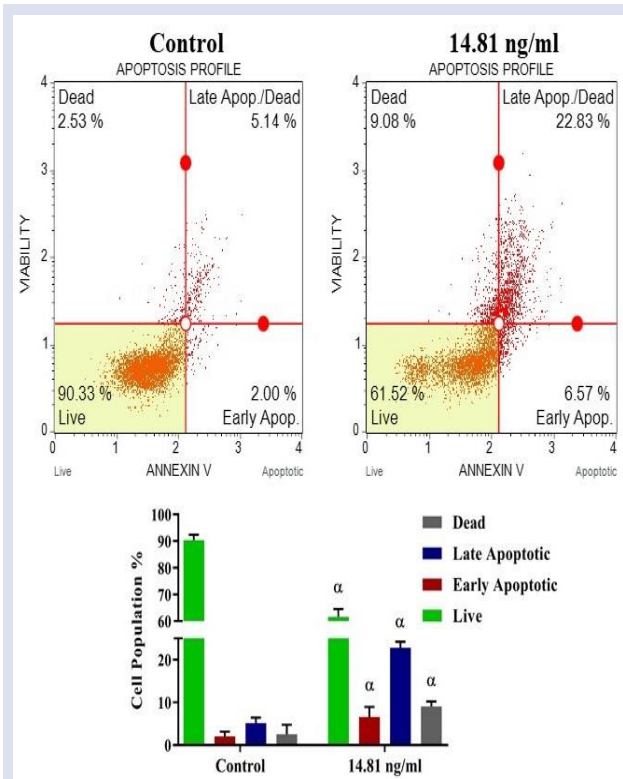


Fig. 2A. Annexin V-determined apoptosis in SNU-1 gastric carcinoma cells after 24 hours of colchicine administration. Cells were exposed to 14.81 ng/mL colchicine and the amount of apoptotic cells was determined by Muse cell analyzer (Merck Millipore) as described above. Early and late apoptotic cell percentages increased significantly in 14.81 ng/mL colchicine-treated cells. Experiments were repeated three times.  $^{\alpha}$ P value  $< 0.01$  vs. Untreated SNU-1 gastric carcinoma cells and 14.81 ng/mL colchicine-treated groups.

Colchicine treatment significantly enhanced the percentage of early and late apoptotic cells in 14.81 ng/ml doses as compared to the untreated cells, according to the Annexin V binding assay in Fig. 2A. The late apoptotic cell population percentage in the control group ( $5.14 \pm 1.27\%$ ) dramatically increased to  $22.83 \pm 1.38\%$  in 14.81ng/ml colchicine treated cells ( $^{\alpha}$ P  $< 0.01$ ). The ELISA measurements were used to assess how colchicine treatment affected the expression of pro- and anti-apoptotic proteins in SNU-1 cells. As can be seen in Fig. 2B, the compound treatment markedly increased Bax ( $^{\alpha}$ P  $< 0.01$ ) and cleaved caspase 3 ( $^{\alpha}$ P  $< 0.01$ ) activities, while anti-apoptotic BCL-2 levels decreased ( $^{\alpha}$ P  $< 0.01$ ).

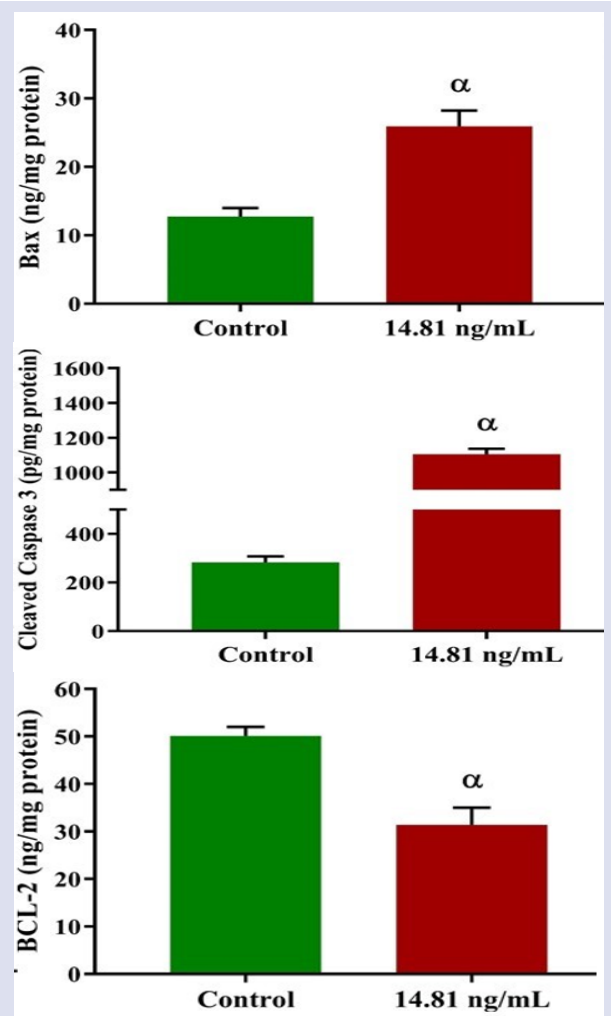


Fig. 2B. Colchicine was administered to SNU-1 cells 14.81 ng/mL for 24 hours, after which the levels of Bax, cleaved caspase 3, and BCL-2 were measured using ELISA kit. Bax and cleaved caspase 3 levels increased significantly in 14.81 ng/mL colchicine-treated cells. BCL-2 levels decreased significantly in 14.81 ng/mL colchicine-treated cells.  $^{\alpha}$ Significantly different when compared to untreated cells ( $^{\alpha}$ P  $< 0.01$ ).

### Discussion

Less than a century ago, gastric carcinoma was probably the most common type of cancer worldwide, according to Ferlay et al[19]. Because of its heterogeneous form and high aggressiveness, gastric cancer continues to be a threat for global health.[20]. For this reason, alternative preventative measures like a balanced diet, early detection, and suitable follow-up treatments lower the frequency of incidents that are recorded[21].

A crucial stage of mitosis that directly affects cancer cell growth is microtubule creation and structure. As a result, targeting microtubules is being considered as a study issue by scientists as they look for new chemicals to develop chemotherapeutics. Microtubule-targeting drugs may also be more effective at reducing the

proliferation of cancer cells due to the higher rate of mitosis in cancer cells.

A well-known and effective microtubule targeting drug is colchicine. It causes mitotic arrest (hyperploidy) by binding to different locations on  $\alpha$ -tubulin, disrupting microtubule functions[[22], [23], [24], [25]]. Colchicine dissociates microtubules into tubulin dimers upon attaching to them, preventing the polymerization of tubulin and disrupting microtubule dynamics [[26], [27]]. Colchicine has long been used to treat gout and FMF(familial Mediterranean fever), but new research has shown that it also significantly inhibits the growth of a number of cancer cell lines, including those from the colon, gastric, liver, and lung[28],[13]. In cancer cells, the rate of mitosis is enhanced, and the microtubules that are created during mitosis are thought to be a prime target for anticancer treatments[29]. We are currently unaware of its cytotoxic effects and cell death mechanisms in stomach cancer. In the current study, colchicine's inhibitory effects on SNU-1 gastric carcinoma cells were examined to gain additional information regarding the potential molecular processes underlying these antiproliferative activities.

There are numerous studies in the literature describing the anticancer activity of different substances with pharmacological action in SNU-1 cells, including amlodipine, verapamil, and propranolol. Parallel to the above, we looked into the antiproliferative effects of colchicine, a drug not typically utilized in chemotherapeutics, on SNU-1 cells in this work. At the beginning, XTT tests were carried out to look into the concentration-dependent cytotoxicity of colchicine on SNU-1 cells. Colchicine strongly suppressed SNU-1 cell proliferation, according to experimental results, and its  $IC_{50}$  value was found to be 14.81 ng/mL for 24 hours.

According to Chen et al., induction of apoptosis with natural product-derived anticancer drugs is a very important strategy to get rid of cancer cells, and promoting apoptosis in cancer cells has an important place in anticancer therapies[[30], [31], [32]]. Hickman (1996) states that apoptosis is well acknowledged to have a significant role in the molecular pathogenesis of cancer and affect how well chemotherapy and radiation therapy work[33]. In the apoptotic process, the proapoptotic protein Bax enables the migration of cytochrome c from the mitochondria to the cytoplasm, and cytochrome c stimulates the apoptotic process[34]. Contrarily, the anti-apoptotic protein BCL-2 blocks the release of cytochrome c from mitochondria and inhibits apoptosis. Increased pro-apoptotic Bax level and decreased BCL-2 level strongly correlated with the mechanism of cell death. The fate of the cell is often determined by the ratio of pro- and anti-apoptotic (Bax and BCL-2) proteins[35]. Caspases are also essential for the apoptotic process, particularly when it comes to the proteolytic cleavage of proteins. According to Bakar-Ates et al. (2020), they are formed by cells as zymogens, and when cells are exposed to cytotoxic substances, they divide to form substrates that induce apoptosis[36]. The

most noticeable caspase is caspase 3, which triggered the endonuclease CAD (Caspase-activated DNase), which results in chromatin condensation and chromosomal DNA destruction[37].

This study used flow cytometry-based testing and ELISA research to determine whether colchicine treatment inhibits cell development as a result of apoptotic changes. Our results demonstrated that colchicine therapy induced apoptosis by enhancing pro-apoptotic Bax, cleaved caspase 3, Annexin V binding, and lowering anti-apoptotic BCL-2 protein in SNU-1 cells. These phenomena provided strong evidence that colchicine can cause SNU-1 cells to apoptose. A recent study by Wang et al. (2017) has shown that cancer cells treated with colchicine frequently induce apoptosis [38]. Colchicine, according to the study of Bhat and Singh (2008), greatly boosted apoptosis in human colon cancer HT-29 cells.[13]. Several studies also discovered colchicine's similar apoptosis-inducing actions in a range of cancer cells[[28], [39]].

## Conclusion

This study shows that colchicine increases apoptosis in SNU-1 cells. Colchicine exhibits high cytotoxic efficacy against SNU-1 gastric cancer cells overall, but more research is needed before it can be used as a new gastric cancer chemotherapeutic drug.

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## Conflicts of interest

There are no conflicts of interest in this work.

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