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Research Article

Determination of the antiproliferative effect of *Folliculj sennea* used as a laxative on CCD-18Co cell line and proliferative effect on DLD-1 and HT-29 cancer cell lines

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Abstract

The most prominent feature of malnutrition that increases colon cancer is the use of laxatives. Most community-dwelling individuals self-manage the condition of bowel-related diseases and do not seek medical advice. Self-management often involves the use of laxative products that can be purchased over the counter from pharmacies and elsewhere. According to the research, most of those who use herbal products do not get enough information about the products they use, and the most important problem is that they do not inform their health consultants (doctors, pharmacists, dietitians, nurses, etc.) about the product they use. Individuals get information about the product they use from transfers such as uncontrolled media channels on the internet, and they reach the product easily. Long-term use of laxatives is predicted to impair healthy colonic function, produce laxative dependence, and damage the enteric nervous system and/or intestinal smooth muscle. It manages colon motility and may increase the risk of other types of cancer, especially colon cancer. In our study, the antiproliferative effect of *Folliculj sennae* plant, which is commonly used as a laxative, known as fasting herb, horseradish herb, and camel eye herb and contains an anthracoid laxative, on CCD-18Co (healthy colon epithelium) cell line and DLD-1 (colon cancer) and HT-29 (colorectal cancer) cancer cell lines, on the other hand, aimed to determine its proliferative effect by MTT analysis.

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Keywords

Folliculj sennae,
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1. Introduction

Colon cancer is the third most common type of cancer worldwide. Colon cancer risk factors that are especially related to lifestyle are smoking, alcohol consumption, obesity, physical inactivity, and some malnutrition and diet factors. It is estimated that approximately 70% of colon cancer cases can be prevented by following a healthy lifestyle, and basic lifestyle factors that can be changed with medical nutrition therapy are at the forefront (Jakszyn et al.,

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2020). Adiposity and lack of physical activity are two other major factors that can contribute to chronic inflammation, and they are highly correlated with diet and are also considered important causes of colon cancer (Clinton et al., 2020). Due to the lack of standardization in the nutrition of individuals, the effects of malnutrition may increase the risk of colon cancer and may hurt the healthy colon epithelium. The most prominent feature of malnutrition that increases colon cancer is the use of laxatives. Most community-dwelling individuals self-manage the condition of bowel-related diseases and do not seek medical advice. Self-management often involves the use of laxative products that can be purchased over the counter from pharmacies and elsewhere. Laxatives have the properties of accelerating or stimulating defecation and are used for many purposes in the community, especially for constipation management. Laxatives generally show this effect by three different mechanisms. Mechanism pathways: (i) enhancing fluid retention through hydrophilic or osmotic mechanisms; (ii) reducing the net absorption of fluid through effects on small and large intestinal fluid and electrolyte transport; or (iii) segmentation is to alter motility by inhibiting (non-impulsive) contractions or by stimulating repulsive contractions (Werth et al., 2020). When looking at the products used as laxatives, herbal products come to the fore. The most important problem in the treatment with herbal products is the use of plants without adequate clinical research as if they are medicine because they are natural, and the unawareness that serious problems may arise as a result of the interaction of herbal products with medicines, among themselves and with food. According to the research, most of those who use herbal products do not get enough information about the products they use, and the most important problem is that they do not inform their health consultants (doctors, pharmacists, dietitians, nurses, etc.) about the product they use. Individuals get information about the product they use from transfers, uncontrolled media channels, and the internet, and they reach the product easily (Uzun et al., 2014). Long-term use of laxatives is predicted to impair healthy colonic function, produce laxative dependence, and damage the enteric nervous system and/or intestinal smooth muscle. It manages colon motility and may increase the risk of other types of cancer, especially colon cancer. In our study, the antiproliferative effect of the *Folliculj sennae* plant, which is commonly used as a laxative and known as fasting grass, horseradish herb, and camel eye herb and contains an anthracoid laxative, on CCD-18Co (healthy colon epithelium) cell line and DLD-1 (colon cancer) and HT- 29 (colorectal cancer) cancer cell lines, on the other hand, aimed to determine its proliferative effect by MTT analysis.

2. Materials and Methods

2.1. Test Item

1 mg/ml main stock was prepared by dissolving the *Folliculj sennae* plant in distilled water in the experimental study. Other concentrations (100; 50; 25; 12,5; 6,25; 3,125 and 1,56 mg/ml) were prepared by serial dilutions of the 1000 mg/ml master stock concentration.

2.2. Cell Culture

In cell culture studies, necessary media and an appropriate environment were provided for the cells to live and reproduce *in vitro.* The medium requirement differs according to the type of cells and their adaptability. Our study used three different media (EMEM, McCOY, DMEM) for three different cell lines (CCD18-Co, HT-29, DLD-1). The media of the cells were kept in an incubator at 37°C, 95% humidity and 5% CO_2 were changed twice a week and the development of the cells was monitored.

2.3. MTT Analysis

When the cells were confluent, they were first washed with PBS (phosphate buffered saline), removed from the flasks using trypsin-EDTA, and the cells taken into the falcon during passage were centrifuged at 16 000 rpm for 10 minutes. 1000 µL of the medium was added to the pellet under the falcon, and the pellet was dissolved, 10 µL of the medium-cell mixture was added to a 0.2 ml tube, and 10 µL of Trypan-blue dye was added. 10 µL of the mixture was taken and spread between the Thoma slide and coverslip. Cells in 16 squares on the Thoma slide were counted using a light microscope. The number of cells was determined according to the formula A x 2 x104. It was taken into 96-well plates and used in MTT analysis. The purpose of using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method; is based on the conversion into formazan crystals by living cells, which determines mitochondrial activity. The cells were seeded onto the plates with the help of a multi-pipe so that the calculated amount of cells was poured into each well of the 96-well plates. The seeded cells were kept in the incubator for 24 hours to adhere to the plate surface. Other concentrations to be studied (100; 50; 25; 12,5; 6,25; 3,125 and 1.56 mg/ml) were prepared with serial dilutions of the master stock concentration prepared with *Folliculj*

sennae used in the experimental study, and *Folliculj sennae* at the concentrations prepared for each cell line was added to the plate in triplicate. . Negative control (cell control), positive control (mitomycin-C) and 1/1000 DMSO concentrations were added to the plate in triplicate and left in the incubator for 24 hours. Since MTT dye is a light-affected dye, 5 mg was weighed for 1 plate in the dark, 1 mL of PBS (phosphate buffered saline) was added to it and 8 mL of medium was added and dissolved by vortexing. The prepared MTT solution was inoculated on the plates and the plates covered with aluminum foil were kept in the incubator for 2-4 hours. At the end of the period, the MTT solution was aspirated and 100 µL of DMSO (100%) was added to each well to stop the reaction. After the plate was kept in the dark for 10 minutes, absorbance values were read spectrophotometrically at a wavelength of 570 nm. The effect of the MTT method on cell density in working cell lines was determined with the concentrations applied with the help of the Microsoft Excel program, and the 50% inhibitory concentration value was calculated.

3. Results

The CCD-18Co cell line; it showed the best effect on the cell viability of the *Folliculj sennae* plant at 100 mg/ml concentration. The % viability activities in the CCD-18Co cell line were determined between 51 and 83% (Table 1) (Figure 1). Therefore, it is thought to have antiproliferative activity in the CCD-18Co cell line.

Table 1. MTT absorbance measurements in CCD-18Co cell line

		% Viability
Cell Control	1,0306	100,0000
Mitomycin-C	0,4105	39,8312
100	0,5289	51,3196
50	0,5884	57,0930
25	0,6257	60,7122
12,5	0,7365	71,4632
6,25	0,6368	61,7892
3,125	0,8625	83,6891
1,5625	0,7325	71,0751

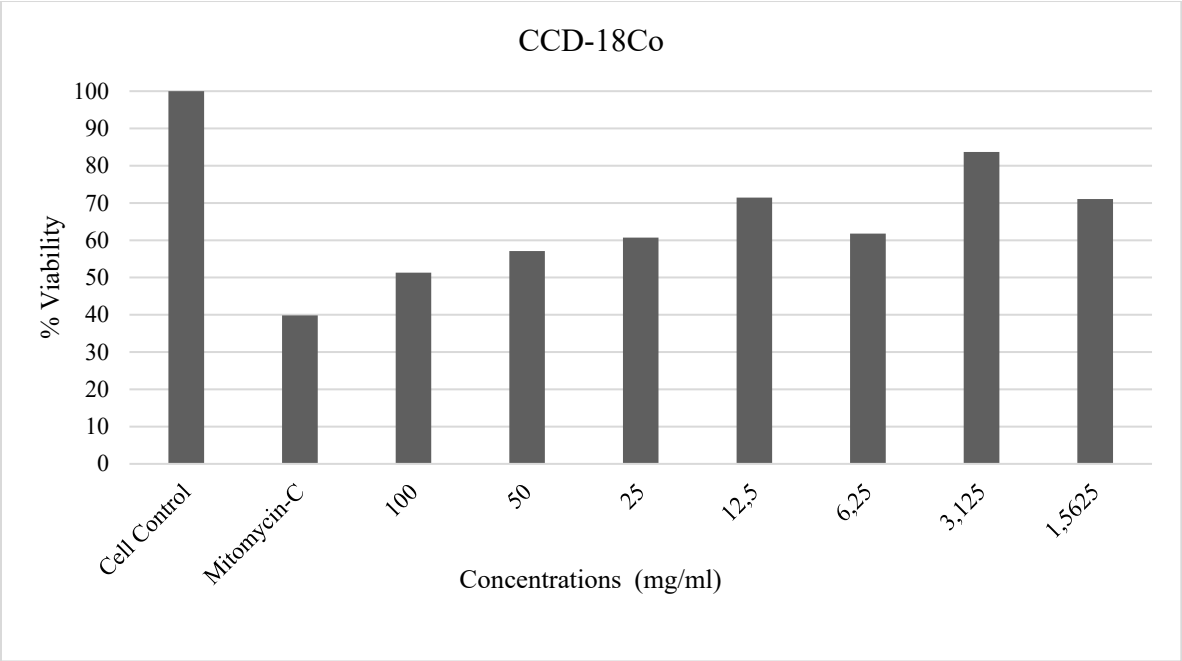

Figure 1. Comparison of percent viability of concentrations in CCD-18Co cell line

The effect of the *Folliculj sennae* plant on cell density was read with a spectrophotometer using the MTT method, the percent viability curve was determined with the help of the Microsoft Excel program, and the 50% inhibitory concentration value (IC_{50}) was calculated with a bar graph and a logarithmic slope line was drawn. The 50% inhibitory concentration (IC_{50}) value from the logarithmic slope line was determined as 67,4 mg/ml.

The DLD-1 cell line; It showed a high effect on the cell viability of *Folliculj sennae* at all concentrations (Table 2) (Figure 2). Therefore, it is thought to have proliferative activity in the DLD-1 cell line.

Table 2. MTT absorbance measurements in DLD-1 cell line

		% Viability
Cell Control	1,4060	100,0000
Mitomycin-C	0,6499	46,2233
100	4,4956	319,7440
50	4,5002	320,0711
25	6,0000	426,7425
12,5	5,3000	376,9559
6,25	6,0000	426,7425
3,125	6,0000	426,7425
1,5625	6,0000	426,7425

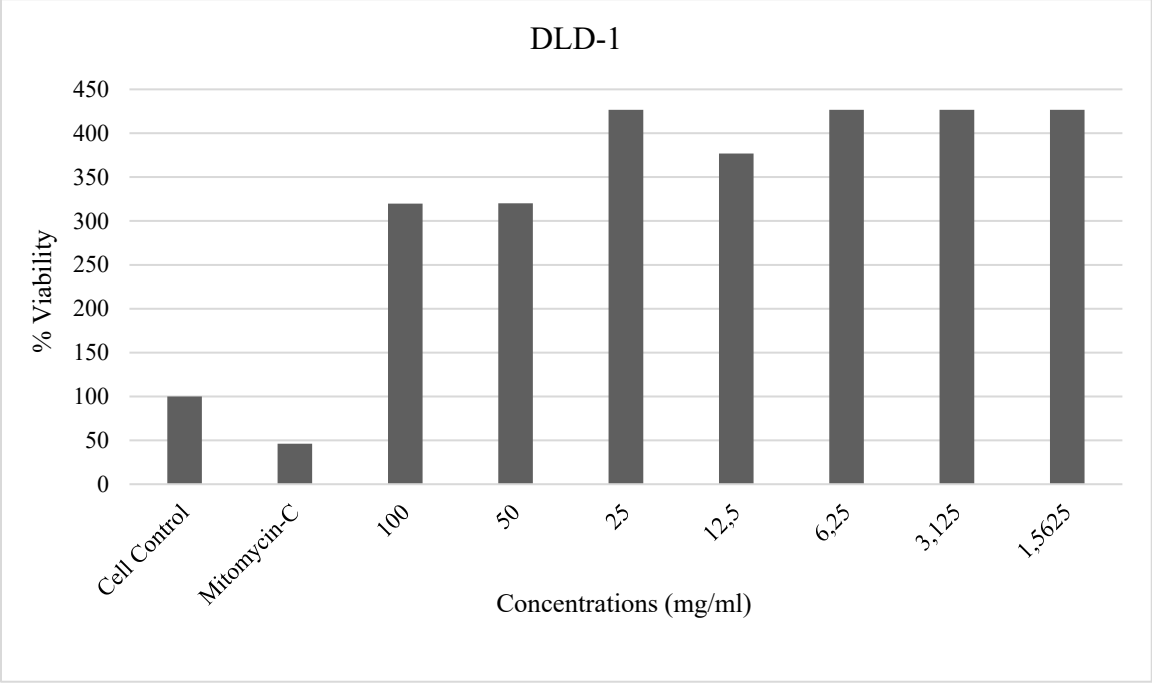

Figure 2. Comparison of percent viability of concentrations in DLD-1 cell line

Since the effect of the *Folliculj sennae* plant on cell density was high at all concentrations by the MTT method, the 50% inhibitory concentration (IC_{50}) value could not be calculated from the logarithmic slope line.

The HT-29 cell line; It showed a high effect on the cell viability of *Folliculj sennae* at all concentrations (Table 3) (Figure 3). Therefore, it is thought to have proliferative activity in the HT-29 cell line.

Table 3. MTT absorbance measurements in HT-29 cell line

		% Viability
Cell Control	1,4060	100,0000
Mitomycin-C	0,8241	58,6131
100	6,0000	426,7425
50	5,5000	391,1807
25	5,1000	362,7312
12,5	5,3000	376,9559
6,25	4,6000	327,1693
3,125	5,1000	362,7312
1,5625	4,2000	298,7198

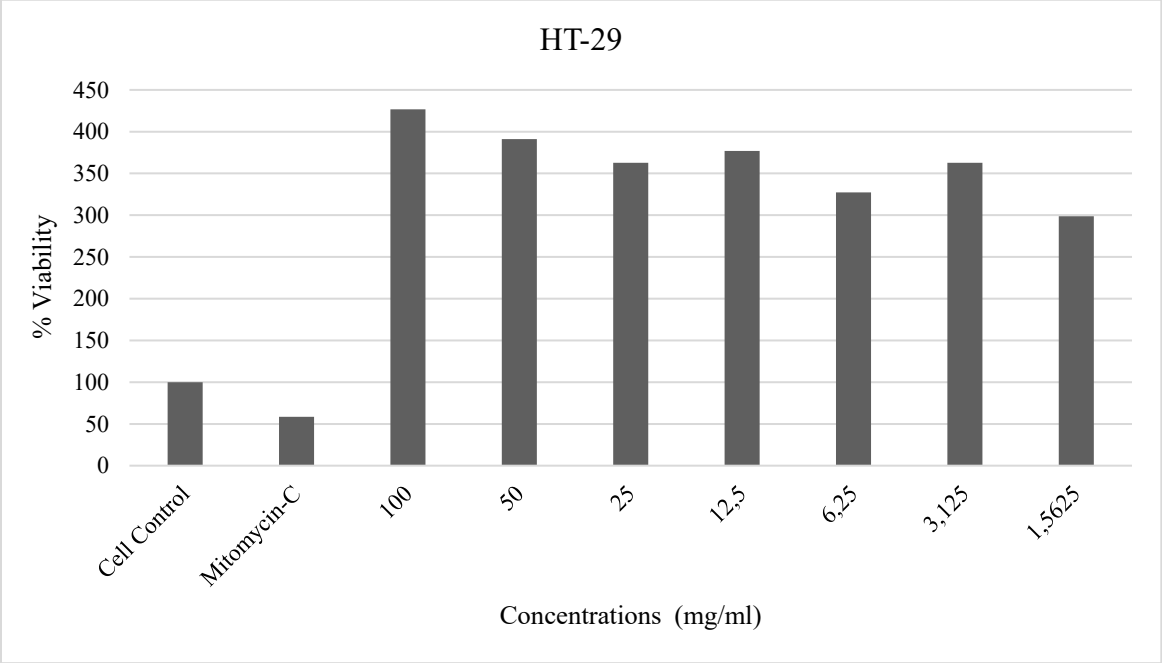

Figure 3. Comparison of percent viability of concentrations in HT-29 cell line

Since the effect of the *Folliculj sennae* plant on cell density was high at all concentrations by the MTT method, the 50% inhibitory concentration (IC_{50}) value could not be calculated from the logarithmic slope line.

4. Discussion

The purpose of our experimental study; the aim in this study is to investigate the proliferative effects of the *Folliculj sennae* plant, which is unconsciously used as a laxative against colon cancer, which is the most common type of cancer worldwide, in colon cancer cell lines, and antiproliferative effects on healthy colon epithelium. In 2018, Citronberg et al. examined the relationship between non-fiber laxative use and fiber-based laxative use and colorectal cancer risk in a multisite International Colon Cancer Family Registry cohort study of 4025 controls. Epidemiological risk factor questionnaires were administered to all participants and exposures were determined approximately 1 year before diagnosis for cases and over a comparable period for controls. Known and suspected risk factors for colorectal cancer have been identified, including regular use of laxatives, defined as a laxative intake for more than one month at least twice a week. People who reported that they regularly used non-fiber-based laxatives were at a significantly higher risk for colorectal cancer than those who reported that they never used laxatives (Citronberg et al., 2018). Among 65,838 women without cancer, the ACS guidelines were associated with a 61% lower risk of colorectal cancer-specific mortality. Among women who survived cancer in 2017, women who had diets consistent with American Institute of Cancer Research guidelines had a 20% lower risk of death over the study period. In 1 of the other studies examining the post-diagnosis diet quality score, women who had diets consistent with the Healthy Eating Index after breast cancer had a 26% lower risk of death over the study period (George et al., 2014). Emodin is an anthraquinone stimulant laxative and is used to treat constipation, and to use it effectively in the treatment of constipation, understanding its mode of action and potential drug targets is of paramount importance. Emodin has provided convincing evidence that it is associated with increased expression of AQP3 by upregulating the PKA/p-CREB signaling pathway by increasing the production and gene expression of AQP3 in the HT-29 cancer cell line (Zheng et al., 2014). Sennoside A is a representative of anthraquinone laxatives and gut microbes may play an important role in the purgative mechanism of sennoside A. Sennoside A caused an increase in the level of epithelial cell proliferation near the tumor tissue. It leads to a possible

malignant change in the proliferation of epithelial cells, suggesting that Sennoside A has a tumor-promoting effect in colitis-associated colonic disease. They observed that sennoside A can disrupt the intestinal mucosal barrier by disrupting the homeostasis of intestinal bacteria and causing the production of intestinal epithelial inflammation, thus promoting the development of colon cancer. Beginning with the key initial link in the occurrence of colon cancer, microbiota-mediated mechanisms linking long-term anthraquinone laxative intake to gastrointestinal inflammation and cancer progression have not been identified. Anthraquinone laxatives and laxative botanicals anthraquinone-containing compounds are frequently used clinically, but the use of anthraquinones for the possible risk development of colorectal neoplasms has long been controversial. Studies show that anthraquinone damages the structure of the colon epithelial tissue. It shows that long-term anthraquinone use has a certain correlation with the development of colon cancer. However, the limited evidence cannot favorably interpret the potential adverse effects of anthraquinone laxatives (Wei et al., 2020). Pseudomelanosis coli is associated with the use of anthraquinone laxatives. This finding has been replicated in animal studies. The genotoxic potential of anthraquinone laxatives has been documented in many in vitro studies. Tanaka et al. observed that rats exposed to a diet containing anthraquinone developed colon carcinoma after 480 days (Tanaka et al., 1990). Several human studies reported a positive relationship between laxative use and colorectal cancer; A prospective case-control study in Germany investigated the risk of anthraquinone laxatives in the development of colorectal adenoma and colorectal carcinoma compared with patients without colorectal neoplasms. They reported no increased risk for the development of colorectal adenomas or colorectal carcinomas. By logistic regression analysis, the odds ratio for colorectal adenomas was calculated to be 0.84, even after adjusting for anthraquinone laxative use time, age, sex, and blood in the stool (Nusko et al., 2000).

5. Conclusion

The cause-effect relationship between unconscious laxative use and colon cancer remains unclear, although several studies have investigated the role of the gut microbiota in the pathogenesis of colorectal cancer. Still, laxatives have been associated with increased tumor detection since first linking high fiber intake to be protective against the development of colorectal carcinoma. In addition to laxative use, various environmental, genetic, and lifestyle factors play a role in the pathogenesis of colorectal cancer. The risk of these factors developing colorectal cancer is unknown. Considering the strong side effects caused by

unconsciously using laxatives by evaluating the findings, the effect of the *Folliculj sennae* plant, which we have witnessed positive effects on DLD-1 and HT-29 cancer cell lines and negative effects on CCD-18Co cell line, on human health should be investigated with large-scale studies. It is thought that the result of our study will make an important contribution to raising the awareness of patients about laxatives by health professionals.

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