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ORIGINAL ARTICLE / ARAŞTIRMA YAZISI

Nutrition and Dietetics / Beslenme ve Diyetetik

The Relationship of Wrong Laxative Use with Constipation and Eating Disorders *in vitro*: Effect on Healthy Colon Fibroblast

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ABSTRACT

Objective: It is estimated that the use of wrong laxatives will increase the probability of developing an eating disorder by affecting the disorder in eating behavior and the physiological disorder of digestion. Long-term use of laxatives is known by many to impair normal colonic function and producing laxative dependence. In our study, it was aimed to determine the effect of the *Folliculj sennae* plant, which is used for laxative and slimming purposes, on the CCD-18Co (healthy colon fibroblast) cell line.

Materials and Methods: The effect of *Folliculj sennae* plant. Its antiproliferative effect on CCD-18Co cell line was determined by MTT test.

Results: According to the concentrations used in the CCD-18Co cell line, the % viability activities were determined between 73 and 54 and the 50% inhibitory concentration value (IC_{50}) was calculated as 47 mg/ml.

Conclusion: It is thought that the *Folliculj sennae* plant, which is used as a laxative and has a negative effect on the CCD18-Co cell line, will make an important contribution by health professionals to raise the awareness of their patients about laxatives.

Keywords: Constipation, Eating disorder, Laxative, CCD-18Co, *Folliculj sennae*

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Ezgi Sinem DAL

Yanlış Laksatif Kullanımının Konstipasyon ve Yeme Bozuklukları ile İlişkisi *in vitro*: Sağlıklı Kolon Epiteli Üzerine Etkisi

ÖZET

Amaç: Yanlış laksatif kullanımı, yeme davranışında düzensizliğe ve sindirimin fizyolojik düzensizliğine etki ederek bireylerde yeme bozukluğu gelişme olasılığını artıracağı tahmin edilmektedir. Laksatiflerin uzun süreli kullanımının birçok kişi tarafından normal kolonik işlevi bozduğu ve laksatif bağımlılığı ürettiği bilinmektedir. Çalışmamızda laksatif ve zayıflama amacı ile kullanılan *Folliculj sennae* (açlık otu) bitkisinin CCD-18Co (sağlıklı kolon fibroblast) hücre hattındaki etkisini belirlemek amaçlanmıştır.

Materyal ve Yöntem: *Folliculj sennae* bitkisinin etkisi CCD-18Co hücre hattı üzerinde MTT testi ile antiproliferatif etkisi belirlenmiştir.

Bulgular: CCD-18Co hücre hattında kullanılan konsantrasyonlara göre % canlılılık aktiviteleri 73 ve 54 arasında belirlenmiştir ve %50 baskılayıcı konsantrasyon değeri (IC_{50}) 47 mg/ml olarak hesaplanmıştır.

Sonuç: Laksatif olarak kullanılan ve CCD18-Co hücre hattı üzerinde olumsuz etkisine şahit olduğumuz *Folliculj sennae* bitkisinin sağlık profesyonelleri tarafından laksatifler konusunda hastalarını bilinçlendirmesi için önemli katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Konstipasyon, Yeme bozukluğu, Laksatif, CCD-18Co, *Folliculj sennae*

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Constipation is defined as a functional bowel disorder characterized by persistent difficult, infrequent or incomplete defecation. Although it is generally considered a mild condition, it can cause many serious complications and deterioration of health-related quality of life (1). Increasing age, female gender, low socioeconomic status, low parental education, low physical activity, stressful life events, physical and sexual abuse, and depression are the factors associated with constipation (2). Chronic constipation affects approximately 10-15% of the population and is among the most common gastrointestinal disorders among primary and secondary health care seekers. It causes a significant health care burden by negatively affecting the quality of life (3). Most community-dwelling adults self-manage the condition 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 (3,4). 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 mobility by inhibiting (non-impulsive) contractions or by stimulating repulsive contractions. The mode of action comes to the fore in the classification of laxatives. There are four main classes in categorizing; bulk-forming laxatives, stool softeners/lubricants, contact/stimulant laxatives, and osmotic laxatives. Although this classification is widely used around the world, it is included in the list of drugs for constipation defined by the World Health Organization's Anatomical Therapeutic Classification (ATC). It shows that especially family, friends and advertisements can be important factors affecting the choice of laxative (3-4-5). In the absence of any gastrointestinal disease that requires specific treatment, it has been shown that constipation should be treated for a short time with laxatives and abdominal pain with pain relieving agents (6). While data on the efficacy and safety of short-term treatment with stimulant laxatives in the adult population support the use of laxatives, there is less evidence to support long-term treatment. Today, in addition to its use for the prevention and treatment of constipation, the prevalence of laxative use is increasing, especially for body weight control, especially in the young population. This can also be an important indicator of early eating disorder (7). It is estimated that the use of laxatives will increase the probability of developing an eating disorder by affecting the disorder in eating behavior, physiological disorder of digestion or psychological disorder. While the lifetime use of laxatives for body weight control among adults is 5%, it is estimated to be between 15% and 62% in those with eating disorders. Those who abuse laxatives can generally be categorized as falling into one of four groups. The first includes patients with eating disorders. The second group consists of generally middle-aged or older individuals who start using laxatives in case of constipation but continue to use laxatives until their bowels become relatively resistant to laxatives. The third group includes individuals who engage in certain types of athletic training, including sports with certain weight limits. The fourth group includes latent laxative addicts who use drugs to cause artificial diarrhea and may have an artificial disorder (5, 8). Patients with eating disorders, who make up the largest percentage of these groups, Anorexia nervosa and Bulimia nervosa frequently abuse stimulant laxatives, and some studies have reported that up to 75% of individuals in this group abuse laxatives. Many eating disorder sufferers use laxatives to induce diarrhea to feel weaker, get rid of unwanted calories, and lose weight. Often times, laxatives are abused after binge eating when individuals believe that laxatives will clear food from the gut before it is absorbed, thereby preventing weight gain. In the case of laxative abuse, greater psychopathology and an increase in clinical severity can be observed in people with eating disorders (5, 9). In addition, failure to respond to a stimulant laxative may reduce the likelihood of responding to a second stimulant laxative (10). Self-administered, inexpensive and readily available laxatives continue to be unconsciously used by adults. In this way, *Folliculj sennae*, a plant containing anthranoid laxative, known as fasting herb, horseradish and camel eye grass, has been used very often as a stimulant laxative for a long time. It is reported that long-term use of these laxatives, which are stimulants that are easily accessible by individuals, primarily carries a risk for colon health. Because stimulant laxatives have traditionally been advocated for short-term use only, and long-term use of these laxatives is estimated by many to impair normal 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 and colorectal can Colon cancer is more common than rectal cancer. Recently, the World Cancer Research Fund (WCRF) and the American Cancer Research Institute (AICR) have concluded in their extensive reports on the scientific literature on diet, physical activity, and cancer prevention that colorectal cancer is mostly preventable with appropriate diets and

associated factors (13).cers. Colon and rectal cancers are the third most common type worldwide (11-12). Recently, it has been considered that long-term laxative use may be an important risk factor for healthy colon fibroblast (CCD-18Co), with a large increase in the risk of constipation, colon and rectal cancer. In our study, the effect of *Folliculj sennae* plant, which is used for laxative and weight loss purposes, was investigated by MTT analysis in CCD-18Co (healthy colon fibroblast) cell line.

MATERIAL AND METHOD

Test Compound

Folliculj sennae in May 2022 from a local herbal store in Gaziantep province, Turkey (The reason for this is that people can easily take it from herbalists and dissolve it in water and use it by drinking). *Folliculj sennae* plant was dissolved in distilled water and 1 mg/ml solution was obtained.

Cell Source

The cell line CCD-18Co (healthy colon fibroblast) (ATCC® CRL-1459TM) purchased from ATCC (American Type Culture Collection) by Gebze Technical University Chemistry Department was used.

Cell Culture

CCD-18Co (healthy colon fibroblast) cells were fed with EMEM (Eagle's Minimum Essential Medium) medium prepared by adding 10% FBS (fetal bovine serum), 1% penicillin-streptomycin and 1% sodium pyruvate in 25 mm^2 culture flasks. The media of the cells, which were kept in a 5% CO2 incubator, 37°C and 96% humidity, were changed twice a week. 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 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 Trypane-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 x10^4.

MTT analysis

According to the determined cell number, they were 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; It is based on the conversion into formazan crystals by living cells, which determines mitochondrial activity. The cells were seeded into the plates (100 µl for each well) with the help of a multipipet, 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 by serial dilutions of the main stock concentration of 1 mg/1 ml (1000 mg/ml) prepared by dissolving *Folliculj sennae* used in the experimental study in distilled water. 100 prepared on the CCD-18Co cell line; 50; 25; 12.5; 6.25; *Folliculj sennae* at concentrations of 3.125 and 1.56 mg/ml were 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, and 1 mL of PBS (phosohate buffered saline) was added to it and 8 mL of medium was added and dissolved by vortexing. The prepared solution was inoculated into 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 antiproliferative effect of the test compound *Folliculj sennae* plant on the CCD-18Co cell line was determined.

Statistical Evaluation of Data

The values of the differences between the data were statistically analyzed by selecting the most effective doses (triple replicate) of the MTT test (25-12.5-6.25 mg/ml) in CCD-18Co cell line. Results were determined as ID50 ± SE (standard error of mean) for the CCD-18Co cell line. When the findings are evaluated statistically; Cell viability concentrations of compounds with CCD-18Co cell line were found to be statistically significant ($p < 0.005$) (Table 1) (Figure 1).

Table 1: Statistical data of Folliculj sennae plant in CCD-18Co cell line

Cell line	Concentration (mg/ml)	ID50 (µM) ± SE
		Folliculj sennae
CCD-18Co	25	1.0431±0.3318
	12.5	1.0515±0.3276
	6.25	1.0924±0.3178

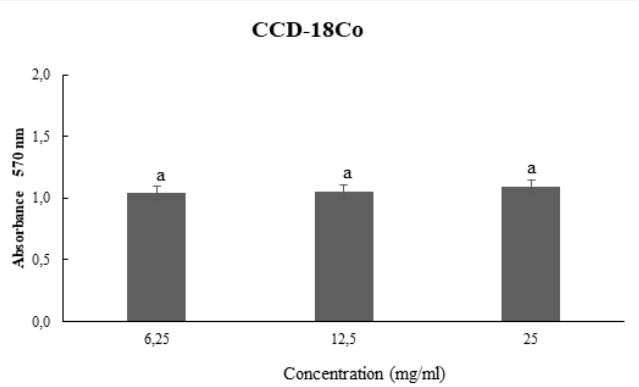


Figure 1. *Statistical analysis of Folliculj sennae plant in CCD-18Co cell line*

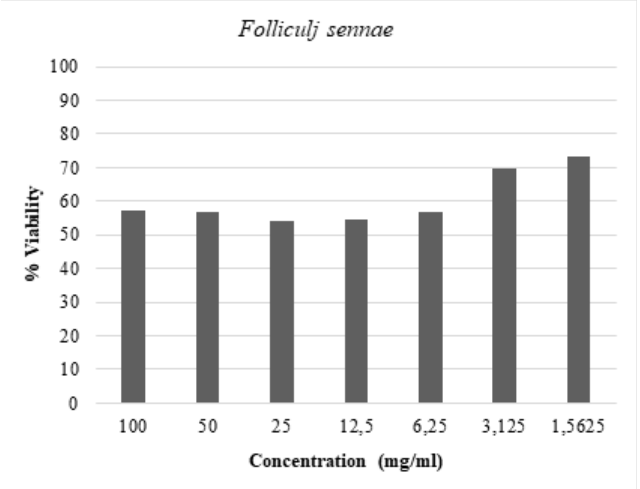


Figure 2. *Comparison of percent viability of concentrations in CCD-18Co cell line*

RESULTS

MTT Analysis Results

In this study, the in-vitro antiproliferative activities of the plant *Folliculj sennae*, which is expected to show potential antiproliferative activity on healthy colon fibroblast, were investigated.

Table 2. MTT absorbance measurements in CCD-18Co cell line		
Cell Control	1.9260	100
(+) Control	0.6070	31.5161
100 mg/ml	1.1043	57.33645
50 mg/ml	1.0970	56.95742
25 mg/ml	1.0431	54.15888
12.5 mg/ml	1,0515	54.59502
6.25 mg/ml	1.0924	56.71859
3.125 mg/ml	1.3448	69.82347
1.5625 mg/ml	1.4149	73.46314
DMSO	1.6446	85.38941

The effect of *Folliculj sennae* plant on cell viability in vitro showed the best effect at 25 mg/ml concentration when compared to DMSO used as a negative control. According to the concentrations used in the CCD-18Co cell line, the % viability activities were determined between 73 and 54%. The effect on cell density was read with a spectrophotometer by MTT method, and the % viability curve was determined with the help of the Microsoft Excel program, and the 50% inhibitory concentration value (IC_{50}) was calculated as 47 mg/ml.

DISCUSSION

In a study conducted on 277 patients diagnosed with eating disorders in France, 22% of the participants were found to abuse laxatives. In addition, in this study, it was found that patients who had attempted suicide in the last 28 days abused laxatives at a higher rate than patients who did not (14). In a 2017 study conducted on adults with 2295 eating disorders (anorexia nervosa, bulimia nervosa), 25% of the participants were found to abuse laxatives. In addition, it has been observed that patients with anorexia nervosa abuse laxatives at a higher rate than patients with bulimia nervosa (15).

In a study conducted on 102,072 Brazilian adolescents, a strong correlation was observed between many unhealthy habits, abuse of laxatives and self-induced vomiting in both genders (16). Guerin et al. (17) aimed to investigate the relationship between the risk of developing colorectal cancer and benign colorectal neoplasm by classifying patients with and without chronic constipation according to the severity of constipation. As a result of the study, patients with chronic constipation were associated with the prevalence and incidence of colorectal cancer and benign colorectal neoplasm compared to patients without chronic constipation. It has been determined that these risks increase with the severity of chronic constipation. In the study conducted on 1021 people (18) eating disorders, personality disorders and traits, and obsessive-compulsive features were evaluated. Laxative abuse was associated with worse eating disorder and general psychopathology and higher prevalence of borderline personality disorder

(BPD). Also, symptom level analyses revealed that specific features of BPD, including suicidality and self-harm, feelings of emptiness, and anger, were most strongly associated with laxative abuse. In 2018, Citronberg et al. (19) 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 laxative intake for more than one month at least twice a week. People who reported regularly using non-fibre-based laxatives were found to be at a significantly increased risk for colorectal cancer compared to those who reported that they never used laxatives. Cell viability was determined on CCD-18Co (healthy colon fibroblast) cell line of *Folliculj sennae* plant, which is used as a laxative to evaluate its antiproliferative activity. Its antiproliferative effect against CCD-18Co cell line was most effective at 25 mg/ml.

CONCLUSION

Considering the evaluation of the findings and the negative effects experienced by the patients due to constipation and the strong side effects caused by the laxatives used, medical nutrition therapy under the control of a dietitian is important. In our study, the effect of the *Folliculj sennae* plant, which we have witnessed on the healthy colon fibroblast, which is used as a laxative, should be investigated with large-scale studies, and it is thought that the result of our study will make an important contribution to raising the awareness of the patients about laxatives by the health professionals.

DECLARATIONS

Funding

None.

Conflicts of Interest/Competing Interests

None.

Ethics Committee Approval

None.

Availability of Data

Available upon request.

Authors' Contributions

Aybüke Afra KESKİNER conducted this study and wrote the article.

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