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Inhibitory Effect of Medicinal Secondary Compounds on Carcinogenic Cell-Lines

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Abstract

There are various methods used to treat cancer including the use of natural compounds. This paper describes the investigation of the cytotoxic effects of the plant *Senna*'s secondary compounds when exposed to different types of cancer cells. *Cassia angustifolia* (*senna*) is a native plant of Yemen, Somalia and Arabia. It is used medicinally in other parts of the world. The practical experiments carried out aim at showing the rate of growth inhibition of the cancer cells after their exposure to the *Senna* extracts. The hypothesis was: *Senna* would inhibit the growth of the cancer cells. The investigation was specifically viability studies and growth analysis of tumor cell lines. Observations were measured and recorded to determine the effectiveness of the varying *Senna* concentration on the specific tumor cells. The first group of experiments carried out exposes the *Senna* extractions to Lung cancer cell line A549. The growth analysis of Lung Cancer Cell line A549 after a "24-hours" exposure period shows inhibition of the growth at concentrations: 2.14, 1.1, 0.5, 0.3, 0.1, 0.07, and 0.03 mg/ml. The percentages of the growth are 0.0, 1.3, 5.9, 10.5, 41.6, 98.3, and 98.2 respectively. The second group of experiments exposes the *Senna* extractions to the colon Cancer Cell Line Colo320 and the results also show inhibition of the growth at concentrations that are 2.14, 1.1, 0.5, 0.3, 0.1, 0.07, and 0.03 mg/ml. The percentages of the growth are 1.15, 13.9, 16.5, 19.8, 44.2, 58.1, and 72.6 respectively. Colon Cancer Cell Line SW620 is affected by the *Senna* extractions, as shown by the third group of experiments. The percentages of growth are 8.6, 12.3, 32.7, 46.5, 75.4, 88.6, and 95.8 for each of the concentrations that are 2.14, 1.1, 0.5, 0.3, 0.1, 0.07, and 0.03 mg/ml respectively. The last group of experiments indicates the growth inhibition rate of the *Senna* on the Prostate Cancer Cell Line PC3. All these cells are affected by the compounds of *Senna* that decrease their growth rate. The percentages of growth are 18.4, 44.3, 75.2, 95.0, 112.1, 115.6, and 105.8 for each of the concentrations that are 2.14, 1.1, 0.5, 0.3, 0.1, 0.07, and

0.03 mg/ml respectively. When comparing the two colon cancer cell lines, Colo320 was inhibited more than SW620.

Subject Areas

Bioengineering

Keywords

Senna, Natural Compounds, A549 Lung Cancer, Colo320 Colon Cancer, SW620 Colon Cancer, PC3 Prostate Cancer, Anticancer

1. Introduction

Cancer can be defined as a special kind of illness that occurs when the cells in a given part of a body begin to grow out of proportion than is normal. Currently, in the United States, this disease is the second leading cause of death [1]. Human beings over history have had tumors and remedies have been implemented, and others continue to be performed and studied over time [1] [2]. Most of the anti-cancer agents being used in clinics now worldwide are either natural or derived from natural products. These natural products include plants, animals and micro-organisms that are of marine origin [2]. The National Cancer Institute (NCI) has conducted large-scale discovery and screening programs which has played the primary role in using natural products as anti-cancer agents. Some of the drugs that come from microbial sources to treat cancer include bleomycin, doxorubicin, mitomycin, and L-asparaginase [2] [3]. Selman Waksman and Boyd Woodruff were the first scientists who discovered the first antitumor antibiotics in the early 1940s. This was done by isolating actinomycin D from Actinomyces antibiotics. This is a compound that acts as an inhibitor of RNA polymerase. It is characterized by anti-bacterial activities. One of the main natural cancer therapeutics is the tubulin-binding agent. Tubulin is soluble and can be referred to as a heterodimer of two molecules. The first molecule is the α-tubulin which consists of six isotypes and the β-tubulin which has seven isotypes [4]. Natural products have contributed majorly to the advancement and development of anti-cancer drugs, Among the 79 FDA approved anti-cancer drugs between 1998 and 2002, only nine were from isolation of natural products directly while 21 of them were from the natural products derivatives [5]. In recent times, there has been a surge in the investigation of the therapeutic characteristics of plants due to their broad medical and economic characteristics, as well as the successful utilization of specific plants in the treatment of human illnesses. Across diverse cultures and communities for millennia, people have recognized plants as a valuable source of bioactive substances that can address numerous diseases, including cancer [6] [7]. There are a good number of plant-derived compounds that are employed successfully in cancer treatment. The most significant example is the vinca alkaloid family which is isolated from the periwinkle *Catharanthus roseus*. The introduction of this plant ex-

tract played a significant role in the cure rates of Hodgkin's and leukemia. The plant extract is capable of doing this because vincristine which inhibits the microtubules assembly induces tubulin self-association into the coiled spiral aggregates. [8]. *Senna* (*Cassia*) plant is an important plant that is used to extract natural compounds and products that are applied in the treatment of tumors. Cassia angustifolia Vahl, commonly referred to as *Senna*, is a species of flowering plant in the Fabaceae family. Originally native to East Africa and the Arabian Peninsula, this plant now thrives in several global regions, including India, Pakistan, and Sudan. This plant species is classified as a shrub or small tree, potentially reaching heights of 2 - 3 meters. It has elongated, thin leaves and produces yellow blossoms. The leaves are composed of several leaflets, arranged in 4 - 8 pairs. Each leaflet measures 1 - 3 cm in length and 0.2 - 0.5 cm in width. Clusters of flowers, measuring around 2 cm in diameter, cluster together at the terminal ends of the branches. The botanical species known as "*senna*" has been utilized historically and currently as a type of laxative that stimulates bowel movements in pharmaceuticals. Approximately 330 species of Cassia exist, and the genus *Senna* classifies about 300 of them according to their evolutionary relationships and we still classify the remaining 30 species as Cassia. [9] [10] [11].

Main Objectives of This Study

1) Determine the growth inhibitory effect of *Senna* (*cassia*) leaf extracts on A549 lung cancer cell lines.

2) Determine the growth inhibitory effect of *Senna* (*cassia*) leaf extracts on Colo320 Colon cancer cell lines.

3) Determine the growth inhibitory effect of *Senna* (*cassia*) leaf extracts on SW620 Colon cancer cell lines.

4) Determine the growth inhibitory effect of *Senna* (*cassia*) leaf extracts on PC3 Prostate cancer cell lines.

2. Materials and Methods

2.1. Extraction of Crude Organic Compounds

Soxhlet, Lyophilizer, and an evaporator were used to extract crude organic compounds from the plants. Vegetation offered access to dried plant leaves. The collected sample of plant leaves were placed in separate 15-millimeter chemical resistant centrifuge tubes. They were frozen for twenty-four hours in liquid nitrogen. After that, they were removed from nitrogen and ground separately using pestle and mortar. To infuse the crude extract, methanol was poured into the mixture and the infusion was distilled for 6 hours using soxhlet. The methanol was then vaporized from the infusion using a rotary evaporator, and the obtained crude extracts were weighed and added to dimethyl sulfoxide to dissolve. The organic extracts were placed in freezer vials and then stored at −20˚C.

2.2. Cell Culture

An evaluation using screening systems and cancerous cell lines on human beings

was utilized to help in the identification of proliferative and cytotoxic activity. The lines were useful in the identification of common cancer types in human beings. Samples that are presented in **Table 1** of the study were obtained from the American Type Culture Collection in Rockville MD. For purposes of preservation, the cells were augmented in RPMI-1640 and attenuated FBS at 10% heat value. However, during the procedure, the 1% penicillin-streptomycin and 2 mM 1-gltamine were not included in the process. Instead, MCF-7 was cultured in low glucose culture media, which is scientifically referred to as DMEM. The concentrate contains 1% penicillin-streptomycin, non-essential amino acids, and 2Mml glutamine. To guarantee the best results, 10% fetal calf serum from Atlanta Biologicals that contains 1 Mm sodium pyruvate and 0.01 mg/ml insulin was added to the DMEM for supplementary purposes. Later, the cells were developed at 37˚C in 5 % CO_2 incubators, which had received the necessary humidity conditions and subcultured twice on a weekly basis to ensure that it attained 80% confluence. Dilutions of the subcultures would take place in the ratios of 1:2 and 1:4 up to a maximum of 40 times.

2.3. Trypan Blue Cell Visibility Test

Finding accurate details about the viability of a cytotoxic effect of a cell has been simplified. One of the commonly used methods is testing the cell membrane. To achieve this in the study, cells were plated on 12 or 24 well plate. About 1×10^5 cells were plated in each well of the 12 well plates or 5×10^4 of the 24 well one. Up to 2 ml mediums are used for the 12 well plate and 1 ml for the 24 well one. In a CO_2 incubator, the inoculated plate is incubated at 37˚C. After 24 hours, the cells were harvested from the culture medium, and washed in phosphate buffered saline. The washed cells would then be exposed for 2 - 3 minutes at 37˚C to 0.25% trypsin-ETDA solution. The treated cells were introduced to trypan blue stains and its live cells were counted. In the long run, they were titrated to a final concentration of 10^3 cells in every 20 μl.

2.4. Dimethyl Sulfoxide (DMSO)

Dimethyl sulfoxide is an essential polar aprotic solvent. For the study, dimethyl sulfoxide was sourced from Sigma-Aldrich. DMSO is an organic compound with sulfur, as well as a colorless and hydroscopic organic liquid with the molecular

Table 1. Cancer cell culture.

Cell Line	ATCC Number	Organ	Oncogenes	Culture Media
A549	CCL-185	Lung	FRA-1	DMEM M-F12
PC3	CRL-1435	Prostates	c-myb	DMEM
SW620	CCL-227	Colon	myc+; myb+; ras +; fos+; sis+; p53+; abl−; ros−; src−	RPMI-1640
Colo320	CCL-220.1	Colon		DMEM

formula C_2H_6OS. The compound DMSO easily dissociates into an array of elements. Despite being an organic compound, it is also hydrophilic, making it a useful control element for the study.

2.5. Tamoxifen

Similar to DMSO, Tamoxifen was obtained from Sigma-Aldrich. Tamoxifen is a steroidal compound, which exhibits anti-cancer properties. It has also been found to be a viable ingredient in the management of advanced estrogen-dependent breast cancer. In the study, tamoxifen was used as a supplementary toxin to compare the inhibition of Echinacea Angustifolia extracts using excerpts from podophyllum peltatum. A portion of tamoxifen stock solution was then dissolved in 1 ml of dimethyl sulfoxide.

2.6. Experiment Procedure

Seven different tubes in two sets were labeled 0 - 6, while the remaining one was not labeled. 15 µl was obtained from each of the serially diluted stock concentrations. The samples were put in corresponding 1.5 ml tubes. 525 µl of the media and 60 µl of the Alamar Blue media were used on each of them. Lids were utilized to cover the contents' vortex for 5 seconds. 20 µl of Alamar Blue was combined with 180 µl of the media in a blank tube. A 96-well plate was obtained, and a sample of 190 µl that was picked from the blank tube was then put in the well that was labeled #A1. 3 different (190 µl) samples of 0 value concentrations were picked and put in F2, G2 and H2 wells. The step was repeated by taking 3 different 190 µl samples of concentration and continuously placing them in F3, G3, and H3 wells. All the samples were vortex at 1.5 µl tubes before being placed in the 96 well plates. The procedure was repeated for the remaining samples.

The next step was the addition of the cells into 7 different tubes. The tubes, each of 1.5 ml, were labeled 0 - 6 while the remaining one was left blank. The obtained samples would then be put into corresponding 1.5 ml tubes. 625 µl of the media was added into each of the 1.5 ml tubes. 60 µl Alamar blue was introduced into the tubes, and 60 µl of the cells were adjusted at 10,000 cells/20µl. Every tube was vortex for 5 seconds. 3 different 190 µl samples of 0 concentrations were put in wells A2, B2, and C2. The steps were repeated continuously by taking 3 different 190 µl samples each of 0 concentration. They were then put in 190 µl samples of concentration 1 and placed in wells A2, B2, and C2. Each tube was vortex for 5 seconds before they were placed into the 96-well plate. The procedure was continuously repeated for the remaining concentration. In the final stage, the 96 well plates were placed in CO_2 incubators at 37˚C for 24 hours. The fluorescence value was recorded with the use of a spectrophotometer.

2.7. Statistical Analysis

Mean ± standard deviations (SD) were the most appropriate formats for presenting the results of the study. T-tests were also utilized for the purposes of cla-

rifying the statistical differences between the correlated samples. The final results of the correlated samples were significantly different.

3. Results and Discussion

3.1. Cytotoxic Effects of *Senna* on Exposure to Cancer Cell Line A549

The growth analysis of Lung Cancer Cell line A549 after a 24-hour exposure period to *Senna* is recorded in **Figure 1**. The t-test indicates an inhibition of the growth of Lung Cancer Call line after a 24-hour exposure period to *Senna* at all concentrations; 2.14, 1.1, 0.5, 0.3, 0.1, 0.07, and 0.03 μg/μl. An increase in the concentration of *Senna* increases the rate of inhibition of growth of the Lung Cancer Cell Line after a 24-hour exposure period to *Senna*. The highest concentration has the highest rate of inhibition while the lowest concentration has the lowest rate of inhibition. An optimum inhibition was observed at 2.14 μg/μl while the minimum inhibition was observed at 0.03 μg/μl. From the obtained findings, it can be said that *Senna* inhibited Lung Cancer Cell line A549 viability at an achievable concentration.

3.2. Cytotoxic Effects of *Senna* on Colon Cancer Cell Line Colo320

The analysis of the Colon Cancer Cell Line Colo320 growth on exposure to *Senna* after 24 hours is recorded in **Figure 2**. The student t-test shows that there is inhibition of the growth of Colon Cancer Cell Line Colo320 after a 24-hour exposure period to *Senna* at all concentrations; 2.14, 1.1, 0.5, 0.3, 0.07, 0.03 μg/μl. The rate of growth inhibition of Colon Cancer Cell Line Colo320 after a 24-hour exposure increases with increasing concentration of *Senna*. The highest rate of inhibition of growth is caused by the highest concentration while the lowest concentration has the lowest inhibition rate. Optimum inhibition was seen at

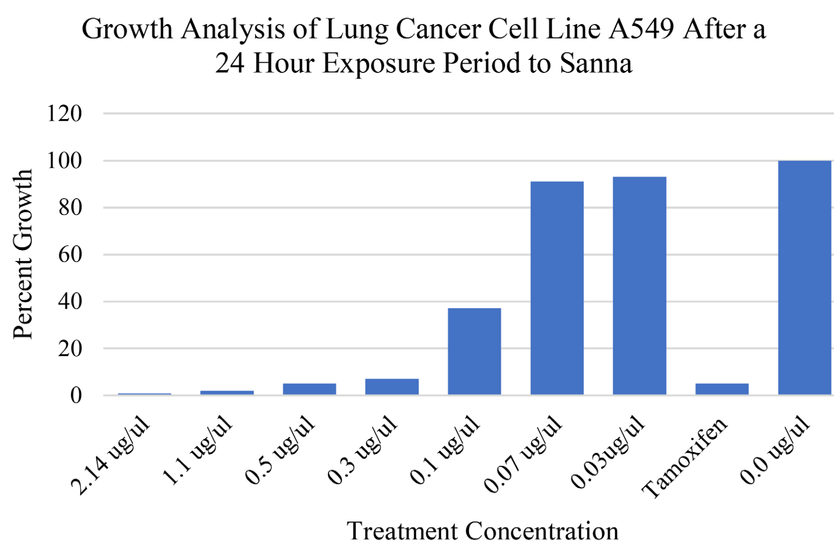

Figure 1. *Senna* leaf extracts significantly inhibited A549 cell viability at some of the concentrations.

2.14 μg/μl while the minimum inhibition was seen at 0.03 μg/μl. From the findings, it can be said that *Senna* inhibited Colon Cancer Cell Line Colo320 viability at an attainable concentration.

3.3. Cytotoxic Action of *Senna* on Colon Cancer Cell Line SW620

The analysis of the growth of Colon Cancer Cell Line SW620 after a 24-hour exposure period to *Senna* is shown in Figure 3. The t-test for the student indicates that the growth of Colon Cancer Cell Line SW620 has been inhibited after a 24-hour period of exposure to *Senna* at all levels of concentrations; 2.14, 1.1, 0.5, 0.3, 0.07, 0.03 μg/μl. The rate of inhibition of Colon Cancer Cell Line SW620

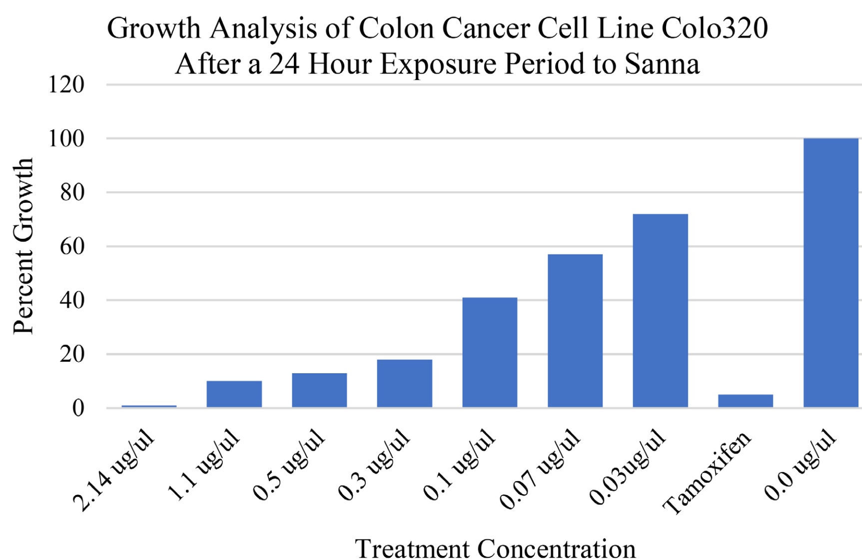

Figure 2. *Senna* leaf extracts significantly inhibited Colo320 cell viability at all of the concentrations.

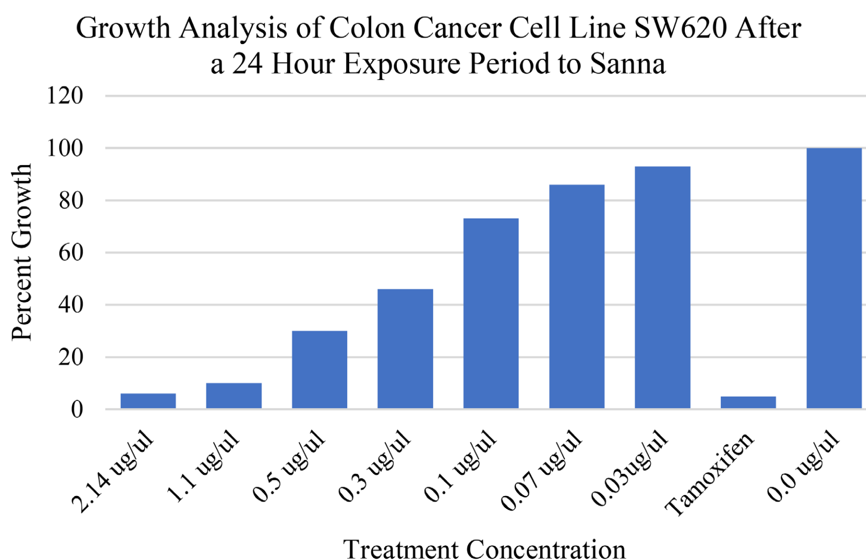

Figure 3. *Senna* leaf extracts significantly inhibited SW620 cell viability at all of the concentrations.

increases with increasing concentration of *Senna*. The highest concentration of *Senna* causes the highest rate of inhibition while the lowest concentration of *Senna* inhibits the growth of the Colon Cancer Cell Line SW620. The optimum inhibition is observed at 2.14 µg/µl while the minimum inhibition is observed at 0.03 µg/µl. As it is seen from the findings, it is clear that all concentrations inhibited Colon Cancer Cell Line SW620 viability.

3.4. Cytotoxic Activity of *Senna* on Prostate Cancer Cell Line PC3

The growth analysis of Prostate Cancer Cell Line PC3 after a 24-hour exposure period to *Senna* is shown in the records displayed in Figure 4. The student's t-test shows that there is inhibition of the growth of Prostate Cancer Cell Line PC3 after a 24-hour exposure period to *Senna* at some level of concentrations: 2.14, 1.1, 0.3 µg/µl. The inhibition rate of Prostate Cancer Cell Line PC3 grows with increasing concentration of *Senna*. The highest concentration of *Senna* gives the highest inhibition rate while the lowest concentration does not inhibit growth rate. The optimum inhibition is seen at 2.14 µg/µl, and the minimum inhibition is observed at 0.3 µg/µl. As seen from the figure, not all concentrations inhibit growth rate.

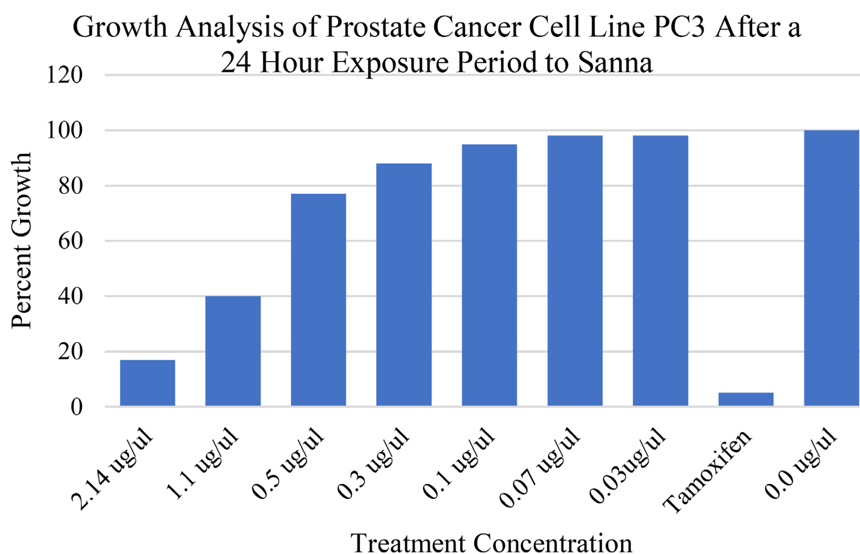

Figure 4. *Senna* leaf extracts significantly inhibited PC3 cell viability at some of the concentrations.

4. Conclusion

In conclusion, the results above imply that the *Senna* plant compounds effectively prevent the growth of all cancer cells. The best result from the experiment shows an inhibition of the growth rate of the cancer cells at all concentrations. The cell viability is reduced, which prevents further growth of cancer. The optimum inhibition occurs at 2.14 µg/µl, while the minimum inhibition is observed at 0.03 µg/µl. The growth rate of the cancer cells is slowly reduced by the increase in the concentration of *Senna* extraction. Senna extraction at its greatest concentration

results in the highest rate of inhibition, but its lowest concentration can still slow down growth.

The use of the *Senna* extraction in the prevention of cancer is explained by the rate at which it inhibits the viability of the cancer cells. *Senna* is introduced to the area affected by cell multiplication in the body as a result of cancer. It acts by reducing the rate at which the cells grow. However, it is not effective in all types of cancers. The cancer cells are different, and the inhibition rate might be slower in some types, whereas in some, it is effective. The effect of the *Senna* on the cancer cells as shown by the above results implies that *Senna* is a suitable anti-cancer.

Conflicts of Interest

The authors declare no conflicts of interest.

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