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MAKING CANCER MORTAL: INVESTIGATING CURCUMIN'S EFFECTS ON CANCER

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1.1 Abstract:-

Curcumin, a polyphenol derived from Curcuma longa, or turmeric, with various medically useful properties as anti-inflammation or anti-oxidation, can be predicted to induce the death of cancer cells through its additional anti-carcinogenic properties. Yet, unlike other cancer treatments today, it should leave healthy, normal cells unharmed since it is used commonly as an everyday cooking spice. Both literature reviews and research conducted within this study indicate that curcumin is capable of implementing its anti-carcinogenic properties along many different biological pathways such as cell cycle regulation, apoptosis, metastasis, and so on without damaging healthy cells. Furthermore, by inhibiting a transcription factor known as NF- κB, curcumin not only induces apoptosis in cancer cells but also prevents the further growth and propagation of cancer cells. In this in vitro study, the effects that curcumin has upon cancer cells are observed and studied in order to explain how curcumin enhances necrosis in cancer cells and to show that curcumin is indeed an inhibitor of cancer cells and their growth.

1.2 INTRODUCTION

Natural plant products have been used throughout human history for many medicinal purposes. Turmeric is one such plant. It is the same spice that led Vasco da Gama to discover the route to India and Christopher Columbus to discover the Americas in the 15th century (Nair).

Turmeric has been used in India for generations in the form of a cooking and healing spice, also "widely used in traditional Indian medicine to cure biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis" (Aggarwal). Turmeric has a natural compound called curcumin, a yellow pigment present in turmeric, which constitutes for 2%–5% of turmeric and contains phenolic hydroxyl groups, giving turmeric some of its many medicinal properties (Aggarwal).

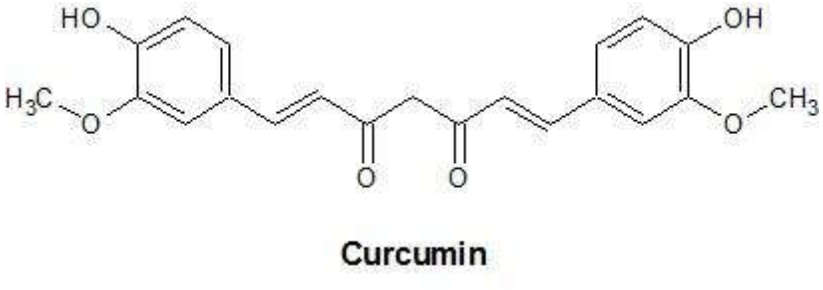


Fig. 1 Chemical Structure of Curcumin

Cancer is a disease in which cells in the body grow out of control. Since curcumin is a polyphenol compound, there are numerous studies that curcumin, when induced on cancer cells, results in apoptosis. It suppresses only the cancer cells and does not harm the healthy cells in the body. Moreover, curcumin is especially effective as a cancer treatment method because it does not harm normal, healthy cells and so, is considerably different from other methods of treatment such as chemotherapy or radiation therapy.

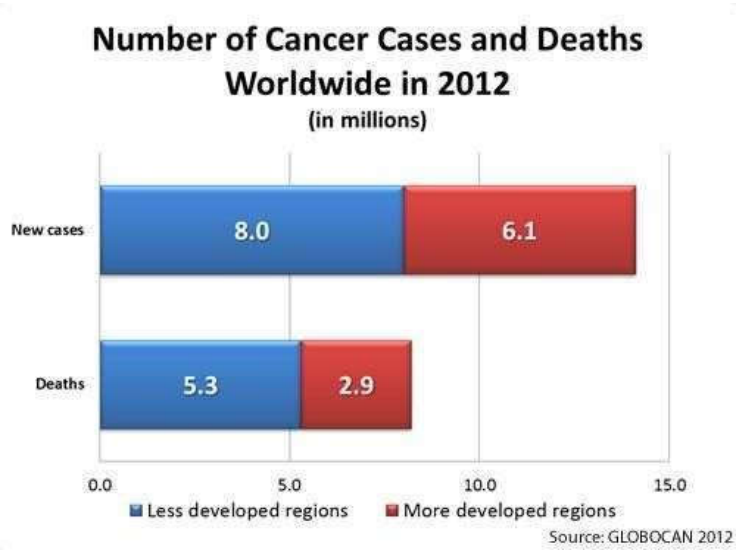


Fig. 2 Cancer Mortality & Diagnosis Rates

Along with its many medicinal properties, one main property is that curcumin can suppress NF-κB. NF-κB is a transcription factor involved in gene expression in cell proliferation, metastasis, and resistance to chemotherapy. Activated NF-κB suppresses apoptosis in many different tumor cells. Curcumin can inhibit the NF-κB signaling pathway. Inflammation is a risk factor for many types of cancers. NF-κB is a mediator of inflammation, and when it remains in the immune system, it is essential to the sustenance of life. However, once it exits the immune system and becomes activated, NF-κB creates havoc in the form of diseases like cancer. It can be induced by many sources of inflammation such as smoking, tobacco, obesity, and so on. Turmeric, or specifically, curcumin, is proven to block NF-κB, delaying or even preventing many chronic diseases, including cancer.

NF-κB is part of a "family of structurally-related eukaryotic transcription factors that are involved in the control of a large number of normal cellular and organismal processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis" (Gilmore). This transcription factor is found in even the simplest of organisms, such as Cnidarians, and thus, has an instrumental role in controlling various functional aspects of immune system and inflammatory responses.

Most transcription factors are regulated by means of some protein or protein kinase. NF- κB activity is regulated mainly through interaction with IκB proteins. In many cells, NF-κB is present in the cytoplasm as an "inactive, IκB-bound

complex" (Gilmore). It is activated only when receives certain extracellular signals from various signal molecules, after which NF-κB can enter the nucleus and commence transcription and gene expression.

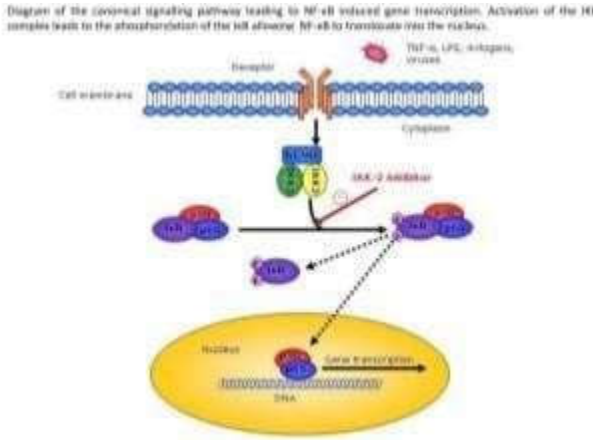


Fig. 3 the NF-κB Pathway

However, once outside the control of the cell, this transcription factor can be incredibly harmful as it causes inflammation, irregularities in the immune system, and possibly the growth and proliferation of cancer. Therefore, it is incredibly important to remain aware of the IκB-NF- κB relationship as curcumin may be targeting that specific interaction. By thoroughly observing the signaling pathway of NF-κB and its activity with and without the presence of curcumin, it is possible to understand, study, and explain the link between curcumin, cancer, and the increasingly important transcription factor, NF-κB.

Based on literature reviews, chemotherapy is, first and foremost, a relatively painful and harmful method of cancer treatment. Administered either intravenously or orally, chemotherapy targets cells within the body that divide rapidly, such as those of the hair follicles and the digestive tract. Hence, normal, healthy body cells are also damaged by chemotherapy. Radiation, on the other hand, is not as painful as it is harmful. While it damages the genetic material of cancer cells, it can also accidentally damage the DNA of healthy cells as well.

Fatigue and skin irritation are additional side effects that the patient must go through. Other current, natural remedies are also in effect. Phytosterols, for instance, are steroid compounds similar to cholesterol in plants. In many studies ranging from 1999 to the present, phytosterols have been associated with decreased cancer risk. While this may be true, phytosterols do provide many side effects, such as bloating, diarrhea, or constipation. Moreover, there is a complex side effect linking these plant sterols as risk factors for cardiovascular diseases.

Telomerase, a eukaryotic ribonucleoprotein complex, assists in the lengthening of telomere lengths in cancer cells. However, as a remedy, many scientists have tried to link various natural plant remedies, including curcumin, to a reduction in cancer cell proliferation. These scientists believe many such plant remedies could target telomerase and hence, reduce metastasis. However, these assumptions may be unfounded because of the extensive complexity of telomeres and telomerase. There are many regulatory processes where telomeres and telomerase are monitored, making it difficult for these plant remedies to directly affect cancer cells' telomere lengths.

The objective of this study is to observe the effect of curcumin (Curcuma longa) on cancer cells and normal cells and to determine if a longer duration will induce cell death in more curcumin treated cancer cells. This study also shows that the transcription factor NF-κB is suppressed by curcumin.

Curcumin should induce cell death in the cancer cells but not harm normal cells. A longer duration of treatment should induce apoptosis in more curcumin-treated cancer cells. Moreover, cancer cells treated with curcumin should show a decrease in NF-κB activity.

1.3 Materials and Methods

Through the course of the experiments done in this study, various human cell lines were used. These cell lines were procured from the American Type Culture Collections (ATCC) and were maintained in Dr. D. Karunagaran's Cancer Biology Laboratory at the Indian Institute of Technology (IIT) in Chennai, India. Specifically, these cell lines are HeLa for cervical adenocarcinoma, SW620 for colon adenocarcinoma, and HEK293 for normal human embryonic kidney cells.

With the help of a designated supervisor, the medium, or the living environment for the cells, was prepared using Dulbecco Modified Eagle's Medium (DMEM). DMEM is a basal medium used to support the growth of various mammalian cells (C.S.). To prepare the medium for both the normal and cancerous cells, the powdered DMEM was added to Milli-Q water, which is a purified, filtered form of water devoid of all traces of salts. Soon after, 3.7 grams of sodium bicarbonate ($NaHCO_3$) per liter of medium was added to neutralize the acidic medium.

When being stored, the medium was placed in a 4□ C environment in the dark.

Before the curcumin treatment process, Dimethyl Sulfoxide (DMSO) was added to each petri dish. For some of these plates, DMSO acted as a control substance. However, for the rest of these plates, DMSO was added because curcumin is insoluble in water but is perfectly soluble in DMSO (Hjorth). The addition of this cell culture reagent allowed the experiments to be conducted smoothly. To begin the experimentation process, the normal cells and cancerous cells

were treated with 3 μL (of 10 μM stock solution) curcumin added to 1 mL of medium (the final concentration of curcumin subsequently becomes 30 μM), which should then be mixed in the medium. All of the curcumin and DMSO was purchased by the IIT Cancer Biology Lab from Sigma Chemicals. Micro-centrifuge tubes, micro-pipettes, and pre-sterile petri dishes were also used throughout the experimentation. The petri dishes containing the cells were incubated in a CO_2 incubator at 37º C, supplied with 5-10% CO_2 for 48 hours. For this study in particular, 5% CO_2 was used.
The cells were then examined after 24 hours and 48 hours respectively under a phase contrast microscope and pictures of the cancerous and non-cancerous cells were taken using the microscope for later speculation.
After testing the previously made hypothesis that curcumin should induce cell death in the cancer cells but not harm normal cells and that a longer duration of treatment should induce apoptosis in more curcumin-treated cancer cells, the next part of the investigation was addressed:

NF-κB.

To determine whether NK-κB activity is low in curcumin-treated cancer cells, real-time PCR is one viable option. By including genes that encode members of NK-κB and IκB families, receptors activating the pathway, and various transcription factors, the activity of NK-κB pathways can be observed as these genes are amplified through real-time PCR. Oligonucleotide probes are labeled with reporter fluorescent dyes and quencher dyes for specific detection ("Real Time PCR"). Compared to regular end-point PCR, real-time PCR is far more effective in specific compound detection, which is one of the main reasons why it was examined in this study. As the reaction progresses in end-point PCR, reagents are consumed because of amplification of genes. Hence, when PCR takes its measurements at the end of the procedure, it is difficult to know whether decreases in amounts of certain compounds were a result of the compounds' activities and behaviors or the result of the amplification process.
Real-time PCR, however allows for detection of the PCR product early in the cycles of the amplification process. Furthermore, real-time PCR allows for the detection of specific targets and products of PCR, such as NF-κB.

1.4 Results

Among the HeLa cervical adenocarcinoma cells, the solely DMSO-treated cells (the controls) grew and proliferated as expected in both the 24 and 48-hour trials. However, the curcumin-treated cells displayed a large reduction in cancer cells on the petri dish in the 24-hour trials. Moreover, in the 48-hour trials, further cancer cell death was evident, demonstrating that longer applications of curcumin treatment further depleted growth of cancer cells.
In the collection of SW620 colorectal carcinoma cells, the DMSO-treated cell plates over trial periods of 24 and 48 hours again demonstrated cancer cell growth and proliferation. Nonetheless, in the curcumin-treated cell plates, the number of living and growing cells decreased exponentially with more reduction apparent in the 48-hour trials than in the 24-hour trials, apparent when considering the corresponding graph in section 1.7. Also, many cancer cell fragments can be observed floating on the surface of the cell culture medium in Figures 5A, 6A,
7A, and 8A, further indicating that curcumin has induced apoptosis in these cancer cells.
For the last batch of cells, the HEK293, or human embryonic kidney cell, cell line was also treated with both DMSO and curcumin for 24 hour trials alone. The 48-hour trial was unnecessary as this cell line is not cancerous. It was observed that the cells in the control group of DMSO-treated cells lived and grew normally. In the curcumin-treated petri dishes, however, curcumin did not induce cell death or in any other way harm these normal cells, as is clear through the HEK293 graph shown in section 1.7. Hence, this further demonstrates that curcumin is far more effective than many other cancer treatments, such as chemotherapy, which destroy or harm normal and healthy body cells.
After qualitative PCR results were collected, that hypothesis was confirmed as well.

Without curcumin treatment, NF-κB levels were at large and the NF-κB activity was prominent as predicted because of the way it interacts with cancer through various processes. After curcumin treatment, however, the activity of NF-κB in the cancer cells was significantly decreased. This shows that, in accordance with the previously formulated hypothesis, curcumin does indeed inhibit the activity of NF-κB in cancer cells and could be one possible way that curcumin is able to have such a detrimental effect on cancer and its proliferation.

1.4.1 Statistical Analysis

Null Hypothesis (H_0: $\mu_1 = \mu_2$):
There is no significant difference in the number of living cancer cells before and after curcumin treatment.

Alternative Hypothesis or Claim (H_A: $\mu_1 \neq \mu_2$):
There is a significant difference in the number of living cancer cells before and after curcumin treatment such that there are significantly fewer cancer cells after treatment.

- p-value: $p < 0.05$
Critical *t* value: 2.447
Calculated *t* value (HeLa): 8.11956
Calculated *t* value (SW620): 3.93865

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

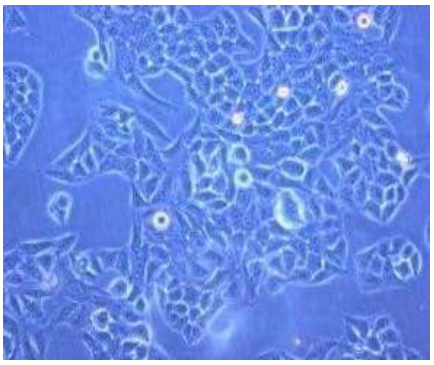
Both the calculated *t* values are greater than their critical *t* values, demonstrating that the null hypothesis may be rejected. The p-value further demonstrates (since it is a low value) that this study provides enough evidence for rejecting the null hypothesis. Hence, there is indeed a significant difference in the number of living cancer cells before and after curcumin treatment such that there are significantly fewer cancer cells after treatment.

1.5 Discussion and Conclusions

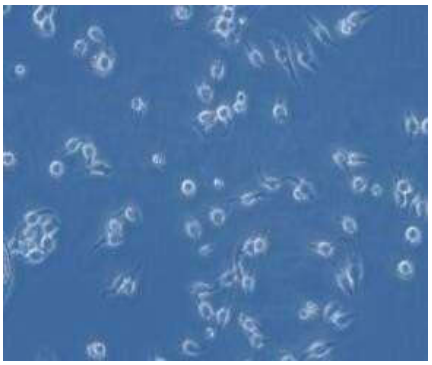
Through observations of morphological changes in shape, color, and structure, it was observed that the non-cancerous curcumin treated cells were adherent, healthy and alive. However, many cancerous cells were observed to be dead and hence, were floating on the surface of the medium. Moreover, longer duration of the curcumin treated cancer cells caused more cell death in the cancer cells. Curcumin had indeed induced cell death only in the cancerous cells and had left the normal cells unharmed.

NF-κB activity and expression had indeed decreased in the presence of curcumin and remained fully active without the presence of curcumin. These findings provide strong evidence that curcumin is able to inhibit the NF-κB signaling pathway. The research performed in this study support the hypothesis that curcumin is an anti- cancerous agent and an inhibitor of NF-κB. Moreover, curcumin may attack cancerous cells, but it does not harm normal cells as shown in the embryonic kidney cells (Figure 9A, 9B). This study provides evidence and data that curcumin is indeed a polyphenol compound which is able to induce cancer cell death by targeting the NF-κB signaling pathway in some way. That Targeting could amount to any amount of overall effect on the cancer cells, whether it contributes very much or very little. Nevertheless, these findings suggest that curcumin is a strong candidate for future cancer treatment in both research and clinical trials as well. In the future, the NK-κB pathways could be analyzed more closely to discover which portion of the pathway curcumin specifically targets. In this way, many other harmful diseases encouraged by that part of the pathway could be treated effectively. Future work could also include an examination of various other cell lines, such as those of lung or breast cancer that are also unfortunately common today.

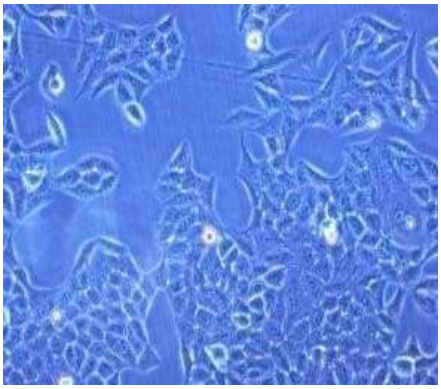
1.6 Figures



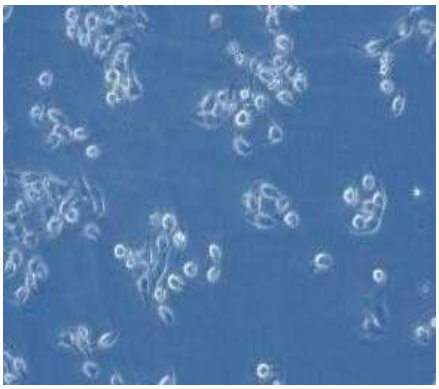
HeLa (Cervical Cancer) – DMSO treated **(Figure 1A)** – 24h



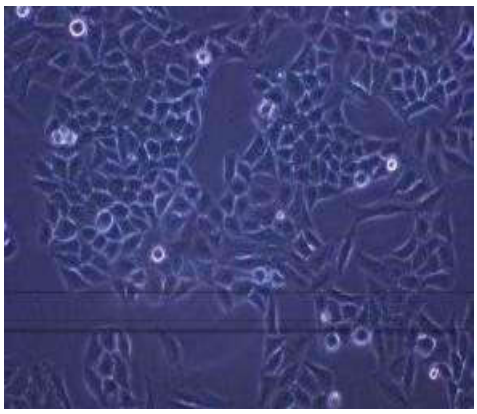
HeLa (Cervical Cancer) – Curcumin treated **(Figure 1B)** – 24h



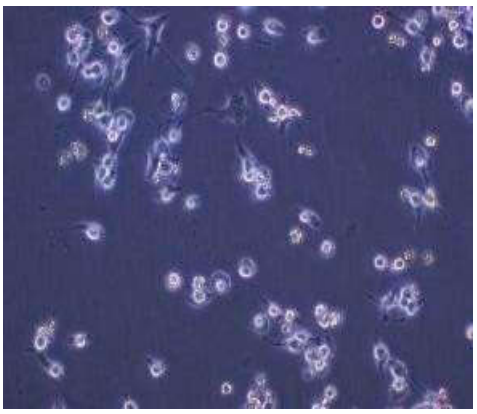
HeLa (Cervical Cancer) – DMSO treated **(Figure 2A)** – 24h



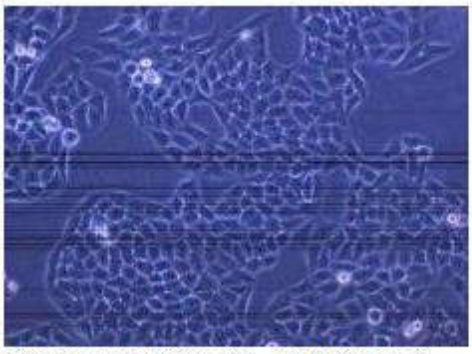
HeLa (Cervical Cancer) – Curcumin treated **(Figure 2B)** – 24h



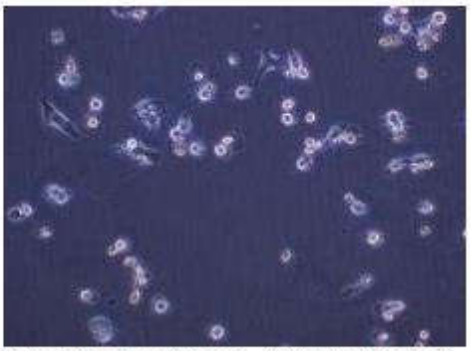
HeLa (Cervical Cancer) – DMSO treated **(Figure 3A)** – 48h



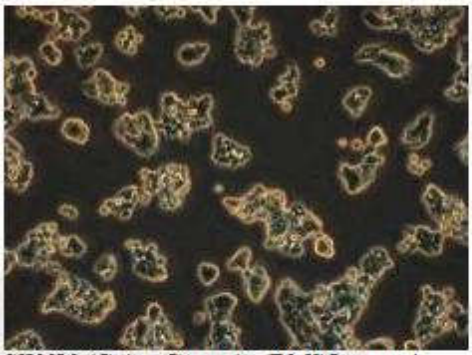
HeLa (Cervical Cancer) – Curcumin treated **(Figure 3B)** – 48h



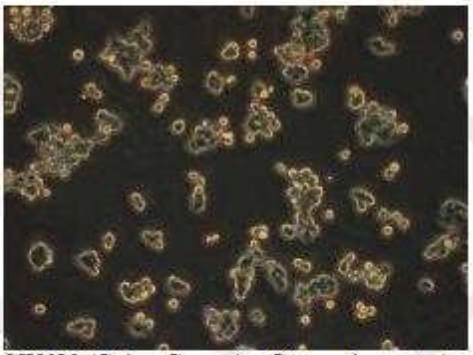
HeLa (Cervical Cancer) – DMSO treated (**Figure 4A**) – 48h



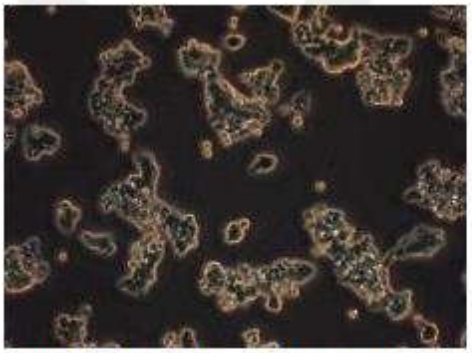
HeLa (Cervical Cancer) – Curcumin treated (**Figure 4B**) – 48h



SW620 (Colon Cancer) – DMSO treated (**Figure 5A**) – 24h

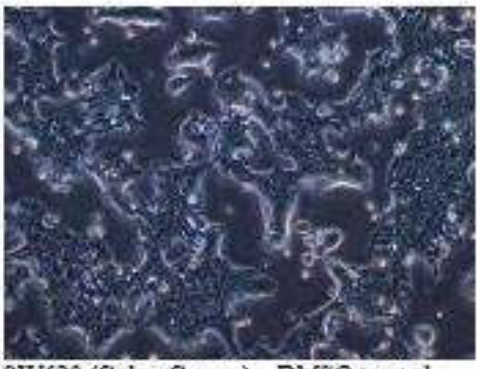


SW620 (Colon Cancer) – Curcumin treated (**Figure 5B**) – 24h

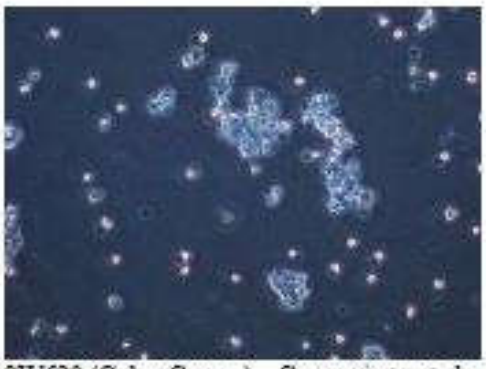


SW620 (Colon Cancer) – DMSO treated (**Figure 6A**) – 24h

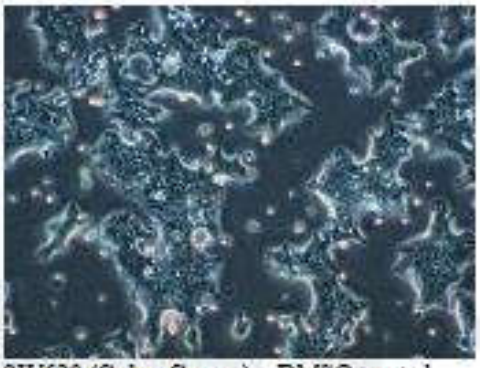
SW620 (Colon Cancer) – Curcumin treated (**Figure 6B**) – 24h



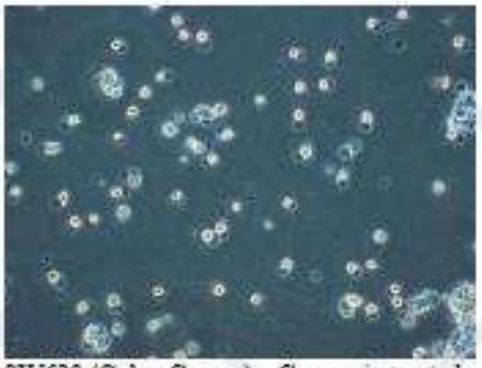
SW620 (Colon Cancer) – DMSO treated
(**Figure 7A**) – 48h



SW620 (Colon Cancer) – Curcumin treated
(**Figure 7B**) – 48h



SW620 (Colon Cancer) – DMSO treated
(**Figure 8A**) – 48h

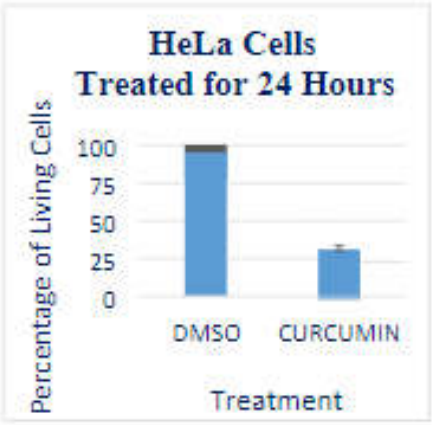


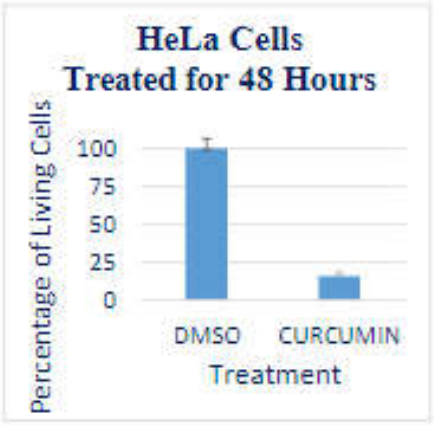
SW620 (Colon Cancer) – Curcumin treated
(**Figure 8B**) – 48h

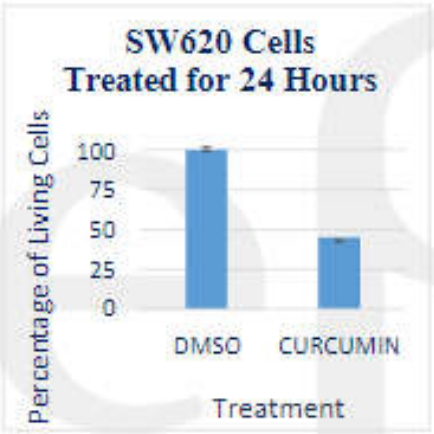
HEK293 (Kidney) – Untreated control
(**Figure 9A**) – 24h

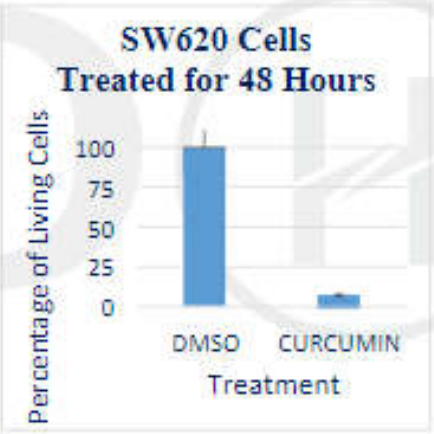
HEK293 (Kidney) – Curcumin treated
(**Figure 9B**) – 24h

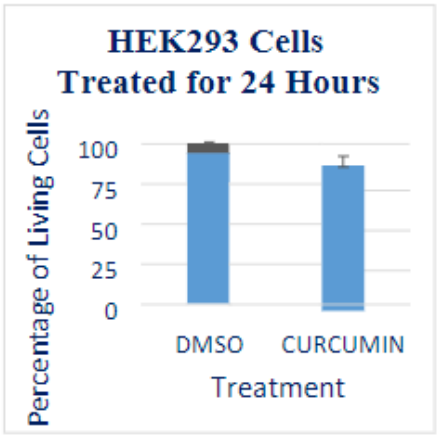
1.7 Graphs











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