Examining the Effects of Phytoestrogens on Ovarian Cancer Cells

A Major Qualifying Project Report submitted to the faculty of
Worcester Polytechnic Institute Department of Biology and Biotechnology
in partial fulfillment of the requirements for the Degree of Bachelor of Science

WPI

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Submitted on April 25th, 2024 to
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Acknowledgments

We would like to thank our advisors, Jill Rulfs and Michael Buckholt, for supporting our journey through this project and fueling our inspiration to grow as young scientists. We admire their genuine passion for the field and unwavering positivity during the difficult moments of unsuccessful laboratory experimentation. Also, a thank you to the Biology and Biotechnology department and Worcester Polytechnic Institute as a whole for providing us with the opportunities for true academic and personal growth.

Abstract

Phytoestrogens have a similar chemical structure to estrogen and can bind the estrogen receptor β. Thus, they have been investigated as potential anti-proliferative therapies in treating estrogen-responsive cancers. This project works to explore this hypothesis through the observation of ovarian cancer cells (OVCAR-3) following treatment with isoflavone rich soybean extracts and Phytoestrogen Solaray®, an over the counter commercial phytoestrogen product. Overall, cell counts from phytoestrogen assays showed mixed results of effectiveness for both treatments. Western blotting and immunoblotting were conducted to determine whether decreases in cell counts correlate positively with markers of apoptosis (Caspase-3) or negatively with markers of replication (PCNA). Due to the inconclusive results, further research and experiments need to be done to understand the true mechanism of action.

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Introduction

Ovarian Cancer

Preliminary stages of ovarian cancer are often asymptomatic. Due to this inability of early detection, as well as limited screening options, ovarian cancer poses a higher threat than other types of cancers (Forstner, 2020). Ovarian cancer is caused by uncontrollable cell growth in the ovaries, which are responsible for egg secretion for fertilization and production of the hormones estrogen and progesterone (Gibson, 2023).

Every one in six deaths is attributable to cancer, making it the second leading cause of death in the world (World Health Organization, 2022). Ovarian cancer, specifically, is the fifth-leading cause of cancer-related fatalities among women, surpassing the lethality of all other female reproductive system cancers. The prevalence of cancer is projected to rise by around 60% in the coming two decades, which will place added pressure on healthcare systems, individuals, and communities (PAHO/WHO, 2023).

Although there are no highly effective screening methods for the early detection of ovarian cancer, treatment methods for later in its development do exist. The most aggressive treatment is surgical removal of the cancer tissue. Radiation and chemotherapy, using electromagnetic waves or strong chemicals to slow or stop cancer cell growth, are other forms of cancer therapy (National Cancer Institute, 2015). Less invasive methods also exist, such as hormonal therapy, in which cancer cells are blocked from receiving the proper growth hormones (CDC, 2023). Our project aims to explore if phytoestrogens, compounds known for their estrogen-like characteristics, can slow down or halt ovarian cancer growth with the hope that they could be implemented as a future preventative therapeutic.

OVCAR-3 Cell Line

The OVCAR-3 cancer cell line was derived from human epithelial cells obtained from a malignant adenocarcinoma in the ovary of a Caucasian woman in her sixties (NIH:OVCAR-3, 2023). The cell line is aneuploid, meaning it lacks several chromosomes and has unidentifiable chromosomes as well. OVCAR-3 cells are positive for both estrogen receptors alpha (ERα) and beta (ERβ), which makes them an ideal model for drug resistance and hormonal therapies (Chao

et Al., 2013). In this study, we observe the effects of different types of phytoestrogens on OVCAR-3 cell proliferation.

Estrogen

Estrogen is a naturally occurring sex hormone that regulates female reproductive development (Johns Hopkins Medicine, 2023). This hormone is formed in the ovaries, secreted through the adrenal glands and adipose tissue, and released into the bloodstream (Delgado & Lopez-Ojeda, 2021). As a steroid hormone, estrogen can cross the plasma membrane and bind to cytosolic receptors ERα and ERβ, located in the tissue of the female reproductive tract, as shown in Figure 2. Once bound, regulation of transcriptional processes that control genomic expression can occur (Fuentes & Silveyra, 2019). When estrogen binds to ERα it activates PCNA to then cause proliferation of the cells to occur. On the contrary, when estrogen binds to ERβ it activates Caspase-3 causing apoptosis (Barone et al., 2008).

Phytoestrogens

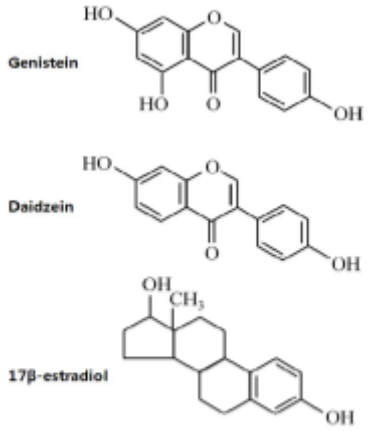

Figure 1. Chemical structures of isoflavones and estrogen (Kim, 2012).

Phytoestrogens are plant-derived compounds that have a similar chemical structure to that of the naturally produced hormone estrogen. Because of their similar chemical structures, as shown in Figure 1, phytoestrogens have been shown to improve postmenopausal symptoms and also hold potential capabilities for cancer treatment. These phytoestrogen compounds are classified into four subgroups: isoflavones, stilbenes, coumestans, and lignans (Desmawati & Sulastri, 2019). For our particular area of research, we will be focusing on isoflavones, which are naturally found in soy products. The structure of these compounds consists of a glycoside bound to a sugar molecule, as shown in Figure 1. Digestion of these compounds results in the release of the sugar molecule, which isolates the isoflavone aglycone (Desmawati & Sulastri, 2019). Aglycones are simply the nonsugar component of a glycoside.

Hydrolysis of these isoflavones into their aglycone form is necessary before full digestion can occur. Genistein has a bioavailability of 10%, meaning that only a small percentage of the compound is circulating in the body and can have a full effect. Daidzein can bind to ERα and ERβ, effectively hindering hormone functions and reducing cell proliferation. The aglycone form of daidzein is either absorbed or broken down into equol and O-desmethylangolensin (O-DMA)

(Alshehri et al., 2021). Equol is shown to have more biological activity and a higher affinity for estrogen receptors, whereas O-DMA has shown no estrogenic activity (Hod et al., 2021).

As mentioned above, it has been hypothesized and demonstrated within numerous studies that phytoestrogens have cancer treatment capabilities. However, this remains controversial because there are conflicting results regarding whether phytoestrogens increase or decrease cell proliferation. For example, in a study by Sakamoto et al., five different isoflavones were tested on MCF-7 breast cancer cells and four out of the five stimulated the G1 to S phase cell cycle transition and, therefore, promoted proliferation (Sakamoto, 2010). Overall, because the research of phytoestrogens and specifically isoflavones' effect on cancer is continuously growing and changing, our group is choosing to focus on the isoflavones within soybeans - genistein, daidzein and glycitein - and their effect on ovarian cancer cells.

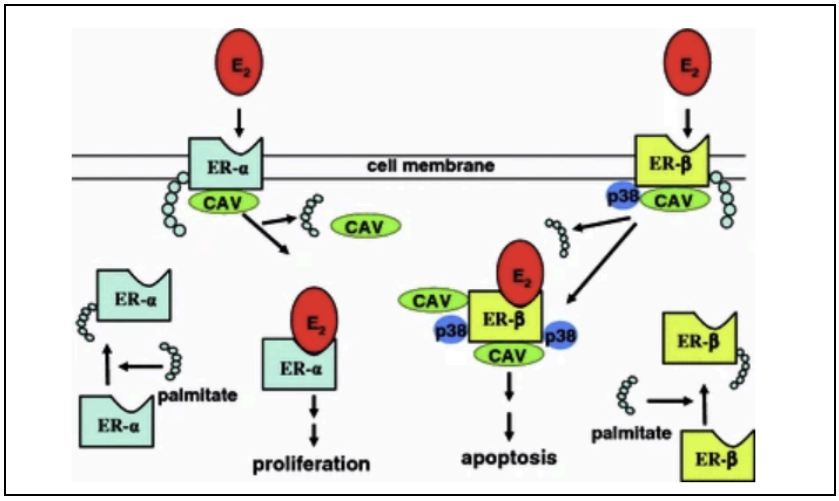

Figure 2. Estrogen receptors ***α*** and **β** pathways (Barone et al., 2008)

Over-the-Counter Product: Phytoestrogen Solaray®

Phytoestrogen Solaray® (Solaray, Inc., Park City, Utah) is an over-the-counter product containing non-GMO soy products such as wild yam, black cohosh, and dong quai, as well as Glycine max, which contains glycitein, daidzein, and genistein. Phytoestrogen Solaray® is mainly used for maintaining a healthy balance of estrogen levels in women, especially for women experiencing menopause (Phytoestrogen, 2024).

Hypothesis

The purpose of this study was to observe the effects of phytoestrogens on OVCAR-3 cell proliferation. To investigate this, we used two distinct sources of phytoestrogens: Phytoestrogen Solaray® and soybeans. We hypothesize that Phytoestrogen Solaray® will decrease cell number in OVCAR-3 cells more than soybeans. This is based on literature that has reported that at high concentrations, phytoestrogens may have an oxidant effect that can induce cell death (Torrens-Mas & Roca, 2020), whereas soy isoflavones have been suggested to counteract oxidative stress by activating the modulation of genes involved in cell proliferation and apoptosis (Fan et al., 2022). However, because anecdotal data shows correlations between high soy diets and a lower incidence of cancer, we chose to observe the impact of soybean extract on the ovarian cancer cells as well (Messina, 2016). Overall, we expect OVCAR-3 cell numbers to decrease. Future work may show that decreases in cell number could be attributed to an increase in apoptosis or a decrease in cell proliferation.

Methods

Phytoestrogen Solaray® and Soybean Extractions

Over-the-counter Phytoestrogen Solaray® (Solaray, Inc, Park City, Utah) tablets and organic soybeans were obtained. The soybeans were ground to a fine powder using a small blender. According to the company website, Phytoestrogen Solaray® contains “non-GMO soy, wild yam, black cohosh, dong quai, vegetable cellulose capsule, maltodextrin, magnesium stearate, ginger root, licorice root, saw palmetto berry and pygeum bark extract” that hold a concentration of “at least 3% isoflavones” (Phytoestrogen, 2022). To extract the isoflavones from both sources, a procedure was adapted from Setchell et al. (2001). 2.5g of the soybean and Phytoestrogen Solaray® powders were extracted using 80% V/V methanol and refluxed at 65°C for 1 hour. After filtration through the Whatman No. 1 filter paper, the volume of the solution was recorded at 90mL for both extracts and the extracts were stored at -20°C.

Cell Maintenance

The OVCAR-3 cells were grown in media containing Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (5000 units/mL of penicillin and 5000 μg/mL of streptomycin) (PS). The cells were plated onto a T75 flask at an initial density of 2.1 x 10^6 cells and incubated at 37°C to adhere and proliferate. The cells were split every 3-7 days to maintain < 70% confluence using 2.5% trypsin, 2.2 mM EDTA. Cell counts and viability were determined using a Cellometer Auto T4 cell counter (Nexcelom Biosciences).

Phytoestrogen Dose Assay

A 12-well plate was obtained and 1mL of media was pipetted into 8 of the 12 wells. Each well was plated with an initial concentration of 0.1 x 10^6 cells of the trypsinized cell solution from the cell splitting procedure. The cells were incubated at 37°C for 24 hours. After the cells were attached, methanol, soybean, or Phytoestrogen Solaray® extracts were added and incubation continued for 24 or 48 hours. The initial plate concentration is shown in Table 1. 1% is the maximum V/V concentration of alcohol that cells can tolerate, therefore, this amount was chosen because methanol was the organic extractant. Additionally, the multiple compounds and concentrations present in the soybeans and Phytoestrogen Solaray® were unknown. The same procedure was run with a 24-well plate to obtain triplicate results (Table 2). Because the percent of treatment compared to the total well volume was different between the first two trials, both the third and fourth trials were run to achieve consistent results (Table 3). However, as described in the results and discussion section of this paper, the results remained inconsistent and immunoblotting was conducted to determine the phytoestrogens' possible mechanism of action within the OVCAR-3 cells.

Table 1. 12-well plate layout for cell counts (8-wells were used). Treatment volumes were 1% of the total well volume.

1	2	3	4
1.00E+05 +	1.00E+05 +	1.00E+05 +	1.00E+05 +

100% Medium	1% Methanol	1% Soybean	1% Phytoestrogen Solaray®
5	6	7	8
1.00E+05 + 100% Medium	1.00E+05 + 1% Methanol	1.00E+05 + 1% Soybean	1.00E+05 + 1% Phytoestrogen Solaray®

Table 2. 24-well plate layout for cell counts for Trial 2. Treatment volumes were 4% of the total well volume.

1	2	3	4	5	6
1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium
7	8	9	10	11	12
1.00E+05 + 2% Methanol	1.00E+05 + 2% Methanol	1.00E+05 + 2% Methanol	1.00E+05 + 2% Methanol	1.00E+05 + 2% Methanol	1.00E+05 + 2% Methanol
13	14	15	16	17	18
1.00E+05 + 2% Soybeans	1.00E+05 + 2% Soybeans	1.00E+05 + 2% Soybeans	1.00E+05 + 2% Soybeans	1.00E+05 + 2% Soybeans	1.00E+05 + 2% Soybeans
19	20	21	22	23	24
1.00E+05 + 2% Phytoestrogen Solaray®	1.00E+05 + 2% Phytoestrogen Solaray®	1.00E+05 + 2% Phytoestrogen Solaray®	1.00E+05 + 2% Phytoestrogen Solaray®	1.00E+05 + 2% Phytoestrogen Solaray®	1.00E+05 + 2% Phytoestrogen Solaray®

Table 3. 24-well plate layout for both Trial 3 and Trial 4. Treatment volumes were 1% of the total well volume.

1	2	3	4	5	6
1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium	1.00E+05 + 100% Medium

7	8	9	10	11	12
1.00E+05 + 1% Methanol	1.00E+05 + 1% Methanol	1.00E+05 + 1% Methanol	1.00E+05 + 1% Methanol	1.00E+05 + 1% Methanol	1.00E+05 + 1% Methanol
13	14	15	16	17	18
1.00E+05 + 1% Soybeans	1.00E+05 + 1% Soybeans	1.00E+05 + 1% Soybeans	1.00E+05 + 1% Soybeans	1.00E+05 + 1% Soybeans	1.00E+05 + 1% Soybeans
19	20	21	22	23	24
1.00E+05 + 1% Phytoestrogen Solaray®	1.00E+05 + 1% Phytoestrogen Solaray®	1.00E+05 + 1% Phytoestrogen Solaray®	1.00E+05 + 1% Phytoestrogen Solaray®	1.00E+05 + 1% Phytoestrogen Solaray®	1.00E+05 + 1% Phytoestrogen Solaray®

Western Blot

The Phytoestrogen Dose Assay procedure for Trials 3 and 4 was replicated at the beginning of this experiment. A 12-well plate was obtained and 1mL of media was pipetted into each of the wells. Each well was plated with an initial concentration of 0.1 x 10^6 cells of the trypsinized cell solution from the cell splitting procedure. The cells were incubated at 37°C for 24 hours. After the cells were attached, methanol, soybean or Phytoestrogen Solaray® extracts were added and incubation continued for 48-hours (Table 3). After incubation, the cells were harvested and triplicates were pooled for cell counting. The four solutions were centrifuged and the tubes were run through three freeze-thaw cycles. Protein concentrations (µg/µL) of each sample were measured using the Thermo Scientific NanoDrop One Spectrophotometer's A280 application. To calculate the maximum protein concentration to load without overfilling the wells, the below equation was used.

$$Loading\ Volume\ (\mu L) = \frac{Desired\ Protein\ Amount\ (\mu g)}{Measured\ Protein\ Concentration\ (\mu g/\mu L)}.$$

The Mini-PROTEAN® TGX™ Precast Gel was loaded with two copies of each treatment along with two copies of a positive control, MCF-7 cells which was the positive control used for

Caspase-3. The orientation of the treatments loaded into the gel is shown in Figure 3. The gel was then cut in half; one half was stained with GelCode™ Blue Stain Reagent (Thermo Scientific™ 24592) and the other half was transferred to an Immobilon P (Millipore) membrane using a Trans-Blot® SD Semi-Dry Transfer procedure.

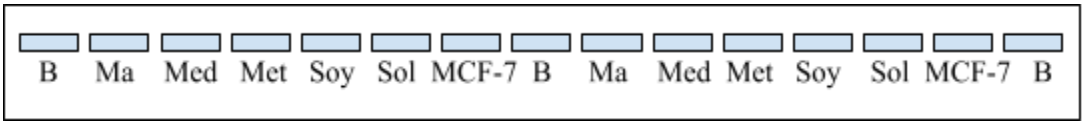

Figure 3. Treatments loaded into the gel. (B: blank; Ma: Marker; Med: Media; Met: Methanol; Soy: Soybeans; Sol: Phytoestrogen Solaray®, MCF-7: Breast Cancer Cells)

Immunoblotting

The membrane was incubated in a blocking solution (5% nonfat dry milk and deionized water) to block non-specific binding sites, gently shaken for 40 minutes and washed with deionized water to remove the excess solution. The first primary antibody solution was made with 10µL of anti-Caspase 3 mouse monoclonal antibody (AB Clonal A17900) and 10mL of TBS + 0.1% Tween. The membrane was gently shaken with the primary antibody solution for 1 hour and then continued to incubate in solution at 4°C for 30 hours. The membrane was then washed in three 10-minute increments with TBS, TBS + 0.1% Tween and TBS (washing procedure). After, the membrane was gently shaken in the second primary antibody solution made with 10µL of anti-PCNA mouse monoclonal antibody (Proliferative Cell Nuclear Antigen, Santa Cruz Biotechnology 25280) and 10mL of TBS + 0.1% Tween for 30 minutes. After the PCNA incubation, the washing procedure was conducted. The membrane was then incubated in TBS for 24 hours at 4°C. The membrane was then incubated in the secondary antibody Goat Anti-Mouse IgG (H+L) Alkaline Phosphatase Conjugated (Invitrogen 31320) at a 1:1000 dilution for 30 minutes while gently shaken and then run through the washing procedure. The substrate tablet (SIGMAFAST™ BCIP®/NBT B5655-25TAB) was dissolved in 10 mL of water, and the membrane was incubated for 30 minutes until blue bands began to form, indicating the presence of protein.

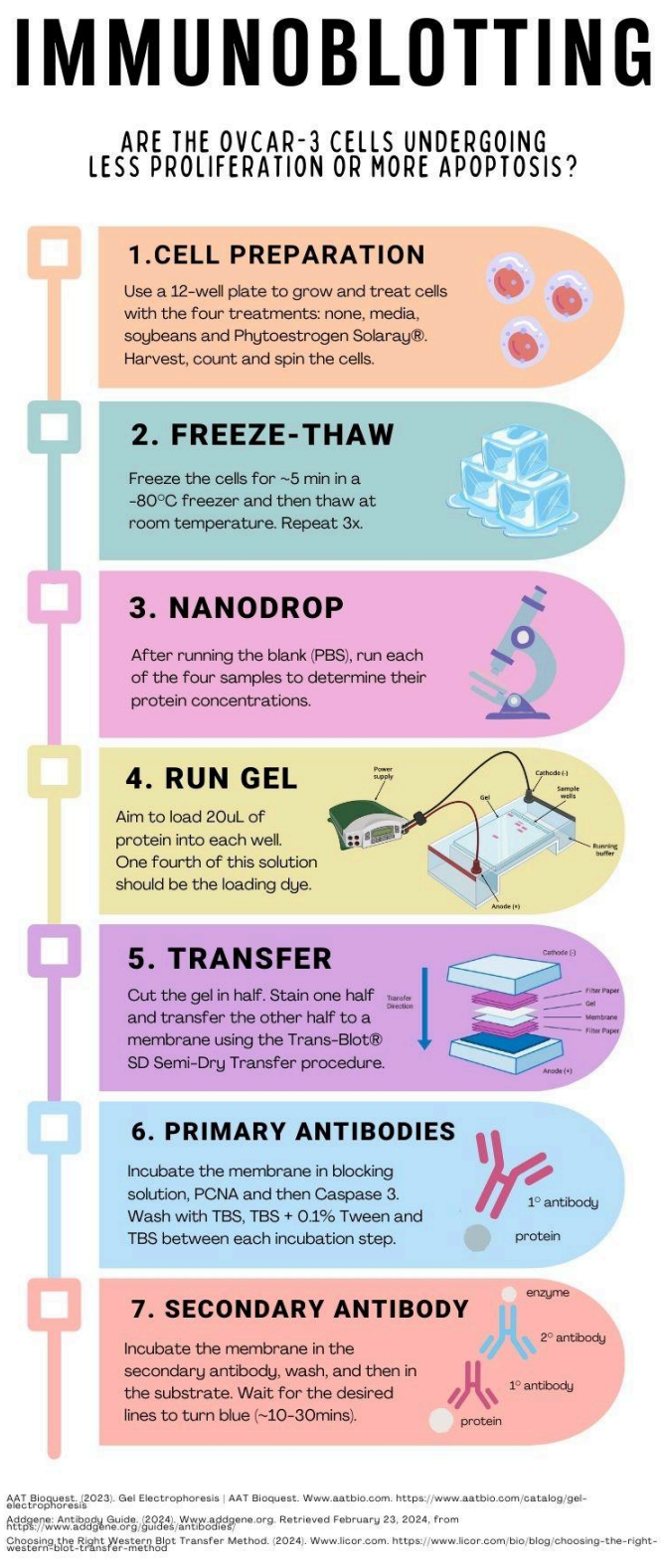

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Figure 4. Flow diagram of the immunoblotting protocol.

Results and Discussion

Effectiveness of Phytoestrogen Solaray® and Soybean Extractions on OVCAR-3 Growth

In order to determine if the soybean and Phytoestrogen Solaray® extracts had an effect on ovarian cancer cell numbers, OVCAR-3 cell counts were examined after 24 and 48-hour treatments. Although at the 24-hour time point there was a decrease compared to the methanol treated control, the soybean extract increased the cell count more than two-fold at 48 hours (Figure 5). This was not an anticipated outcome as the isoflavones within the soybeans were expected to have an antiproliferative effect. However, the Phytoestrogen Solaray® treatment decreased the cell counts by 53.5% at the 48-hour time point, with little difference compared to control at 24 hours. This result aligns with our hypothesis that the Phytoestrogen Solaray® extract will decrease the cell count more than that of the soybeans. However, this experiment represents a single trial with no treatment replicates. Therefore, without multiple data points to average and analyze, we were unable to use these results as the only supporting evidence for our hypothesis.

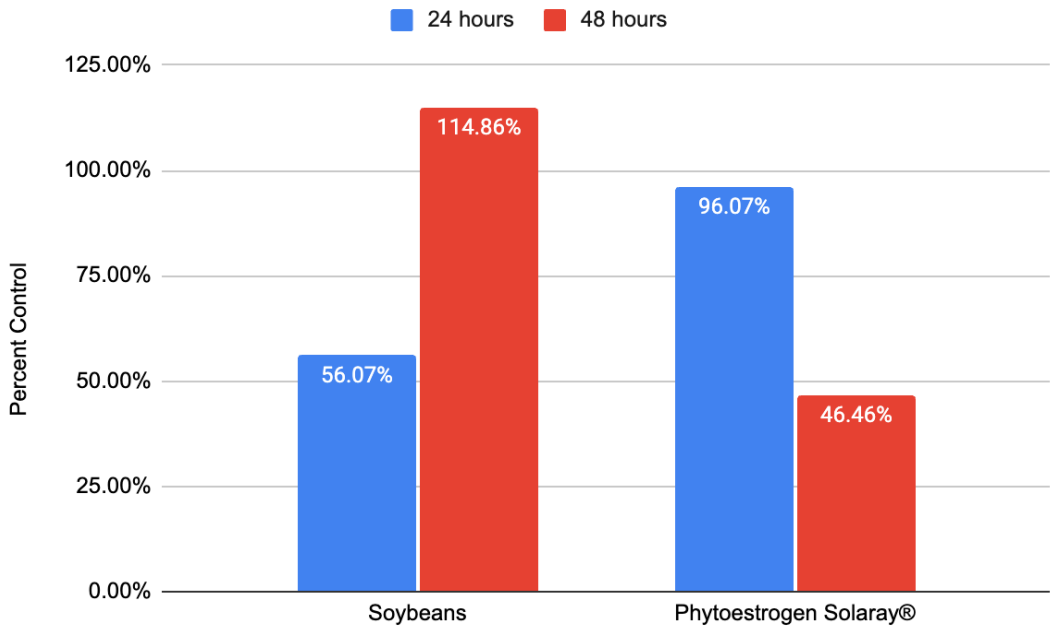

Figure 5. Percent controls of cells treated with 1% (V/V) extracts of soybeans and Phytoestrogen Solaray® compared to methanol in an 8-well assay in a 12-well plate. Each data point represents a single sample for each treatment. N=1

The ineffectiveness of the soybean extract from the 8-well experiment contradicts the results found in the 24-well experiment (see Figure 6). When the experiment was repeated in a 24-well plate, triplicate samples were averaged for each treatment and the control. In this experiment, both extracts increased cell counts at 24 hours, but decreased them markedly at the 48-hour time point. The soybean extracts at a final concentration of 1% (V/V) decreased cell proliferation by 75% compared to the control at the 48-hour mark. A possible explanation for the large decreases in cell proliferation is that the cells were treated with 20µL of extract in the 24-well plate as opposed to that of 10µL in the 12-well plate. Because of the difference in volume between the 12-well plate and the 24-well plate, the 20µL procedure was in fact a 4-fold increase in treatment volume. However, the methanol control was similarly at 4% (V/V) so the difference was not due to exposure to the alcohol. A third trial was conducted to address the disparity in the results from the first two trials (Figure 7).

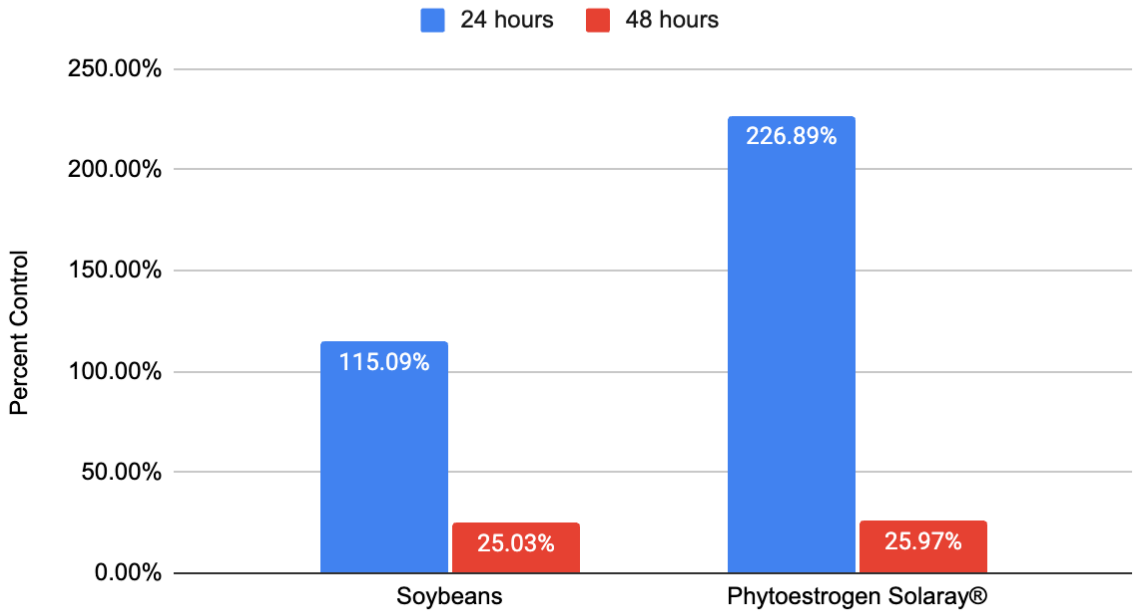

Figure 6. Percent controls of cells treated with 4% (V/V) extracts of soybeans and Phytoestrogen Solaray® compared to methanol in a 24-well assay. Each data point represents the average of triplicate wells for each treatment. N=1

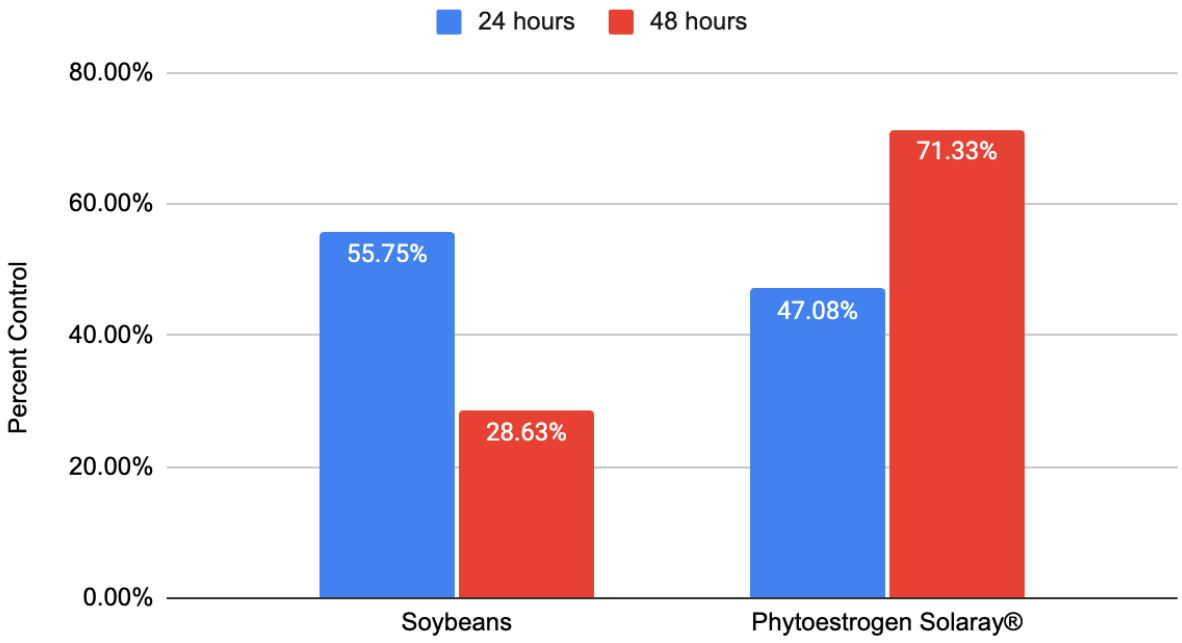

Figure 7. Percent controls of cells treated with 1% (V/V) extracts of soybeans and Phytoestrogen Solaray® compared to methanol in a 24-well assay. Each data point represents the average of triplicate wells for each treatment. N=1

In the third experiment shown in Figure 7, cells treated with either soybean or Phytoestrogen Solaray® extracts resulted in an initial decrease at 24 hours. The soybean treated cells showed a continued decrease in cell number between 24 and 48 hours, while the Phytoestrogen Solaray® treated cells increased in number between 24 and 48 hours when compared to time-matched controls. Since the first trial was not conducted with treatment replicates, a comparison cannot be made between the data in Figure 5 and the data in either Figure 6 or Figure 7. A fourth trial was run to maintain consistency.

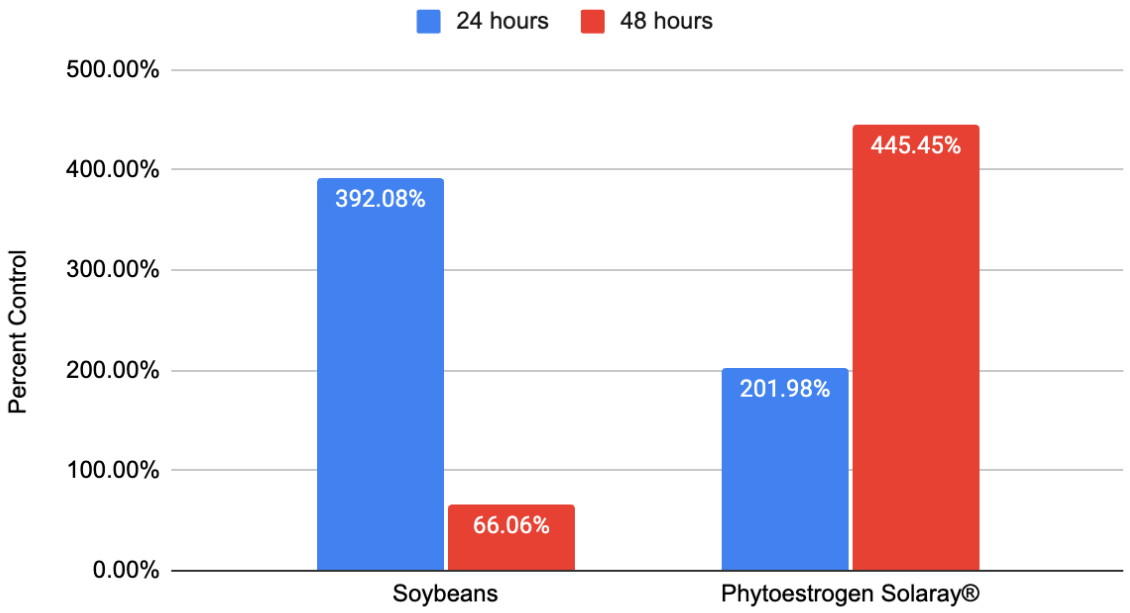

Figure 8. Percent controls of cells treated with 1% (V/V) extracts of soybeans and Phytoestrogen Solaray® compared to methanol in a 24-well assay. Each data point represents the average of triplicate wells for each treatment. N=1

In the fourth experiment shown in Figure 8, the soybean-treated cells increased by 292% at 24 hours but decreased markedly by 34% at 48 hours compared to time-matched controls. However, Phytoestrogen Solaray® treated cells showed increases compared to the controls with a 102% increase at 24 hours and a 345.5% increase at 48 hours. Due to the inconclusiveness of these four trials, immunoblotting was conducted to determine whether the possible decrease in OVCAR-3 cell numbers was due to decreased cell proliferation or increased apoptosis.

Gel Staining

Following the western blot, the gel was stained with GelCode™ Blue Stain Reagent (Thermo Scientific™ 24592) to detect and visualize the proteins. The first time this experiment was performed can be seen in Figure 9a. The results of the staining show an uneven distribution of proteins between each of the wells. The wells containing the methanol treatment and Phytoestrogen Solaray® treatment indicate enough protein and well-defined bands. However, because not all of the wells have the same amount of protein, it would potentially have a negative effect on the outcome of the immunoblot. The variation in protein concentration could be a result of a miscalculation of concentration determined by the Thermo Scientific NanoDrop One Spectrophotometer but this is unlikely. The more likely explanation is that the proteins did not denature in the preparation process of the samples because of the strands of DNA in the samples while loading the gel. Figure 9b shows the gel from the second trial which also indicates the protein concentration in each sample. The results of the gel from trial 2 displayed less protein in each well than before even though adjustments were made in the preparation process to denature the proteins more. The gel also did not show defined protein bands compared to the first trial. The final trial shown in Figure 9c displays three wells of a significant amount of protein however the first two wells of no treatment and methanol did not show enough protein. The gel additionally did not show distinct protein bands suggesting that the electrophoresis did not separate the proteins. The inadequate and uneven amount of protein applied to the gels may have caused the inconclusive results seen in the immunoblots.

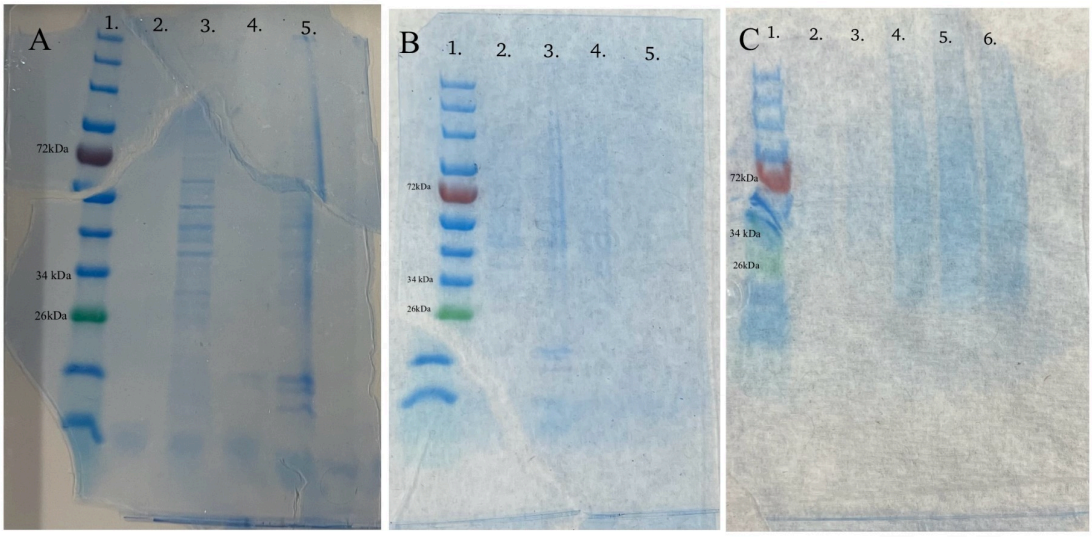

Figure 9. Gel staining from all three trials (1: marker; 2: Media; 3: Methanol; 4: Soybeans; 5: Solaray®; 6: MCF-7; Red Color Protein Stain: 72 kDa; Green Color Protein Stain: 26 kDa)

Immunoblotting

Immunoblotting was conducted to determine if the decrease in OVCAR-3 cell number was caused by decreased proliferation, determined by the presence of the primary antibody PCNA, or apoptosis, determined by the presence of the primary antibody Caspase-3. A visual depiction of this protocol is shown in Figure 4.

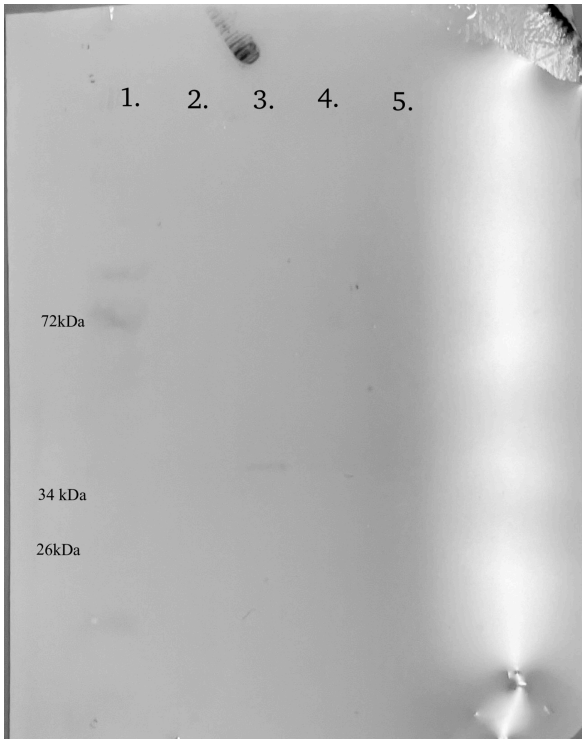

Figure 10. Trial 1 Immunoblot (1: marker; 2: Media; 3: Methanol; 4: Soybeans; 5: Solaray®)

In the first experiment shown in Figure 10, faint bands can be observed in all four treatment wells at 36 kDa indicating the presence of PCNA and that the cells in each were still exhibiting proliferative behavior. The most distinct band was the methanol treatment, this supports the idea that methanol did not have a negative impact on the proliferation of the OVCAR-3 cells. However, there was no evidence of Caspase-3 making it difficult to support or deny the hypothesis. With the extremely faint lines and lack of Caspase-3, a second trial was performed to address the issues.

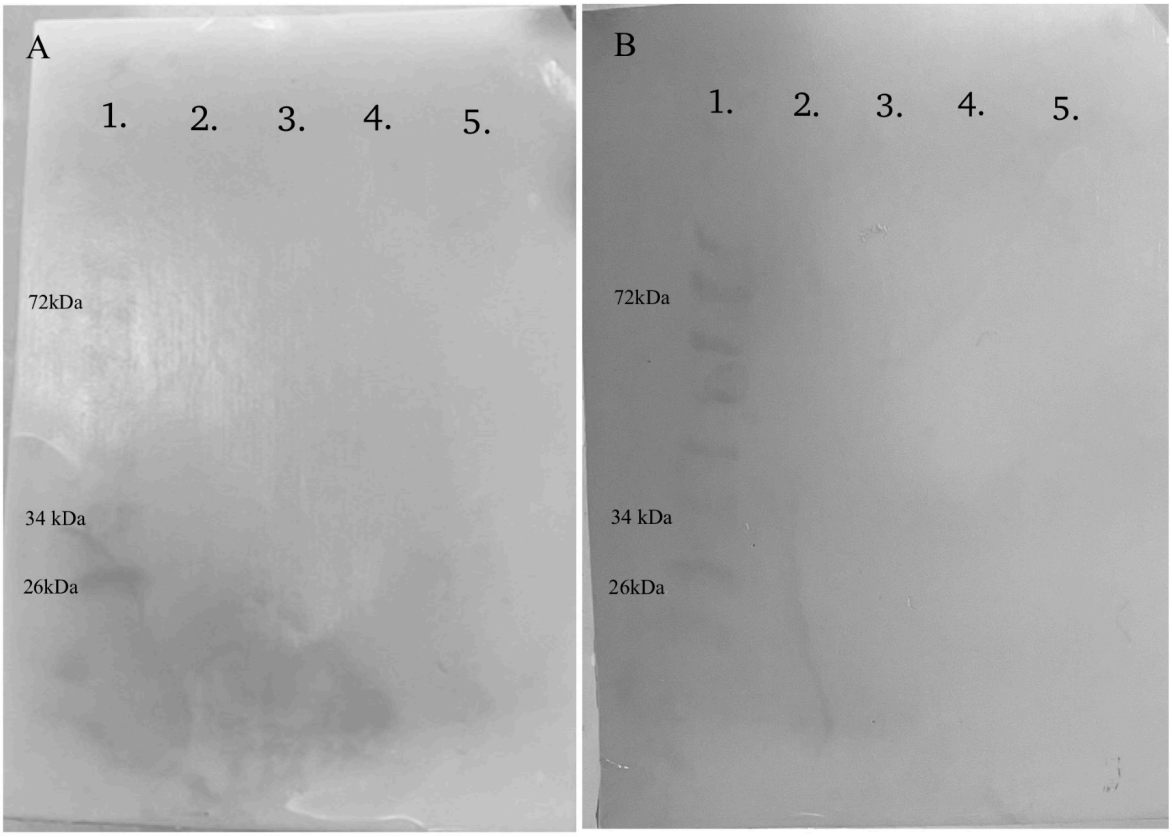

Figure 11. (A)Trial 2 Immunoblot 1 (1: marker; 2: Media; 3: Methanol; 4: Soybeans; 5: Solaray®) (B) Trial 2 Immunblot 2 (1: marker; 2: Media; 3: Methanol; 4: Soybeans; 5: Solaray®)

In the second experiment, the procedure remained the same except for a change in the secondary antibody from Goat Anti-Mouse IgG (H+L) Alkaline Phosphatase Conjugated (Invitrogen 31320) to Rabbit Anti-Mouse IgG Alkaline Phosphatase Conjugated because of the extremely slow reaction time in the substrate. Even with this adjustment, Figure 11 shows the bands on the three different immunoblots were not visible. The purple hue of the membrane may indicate that there were errors in the washing process of the secondary antibody. Due to these errors and inconsistency between the first and second trials, a third attempt was conducted to test the hypothesis.

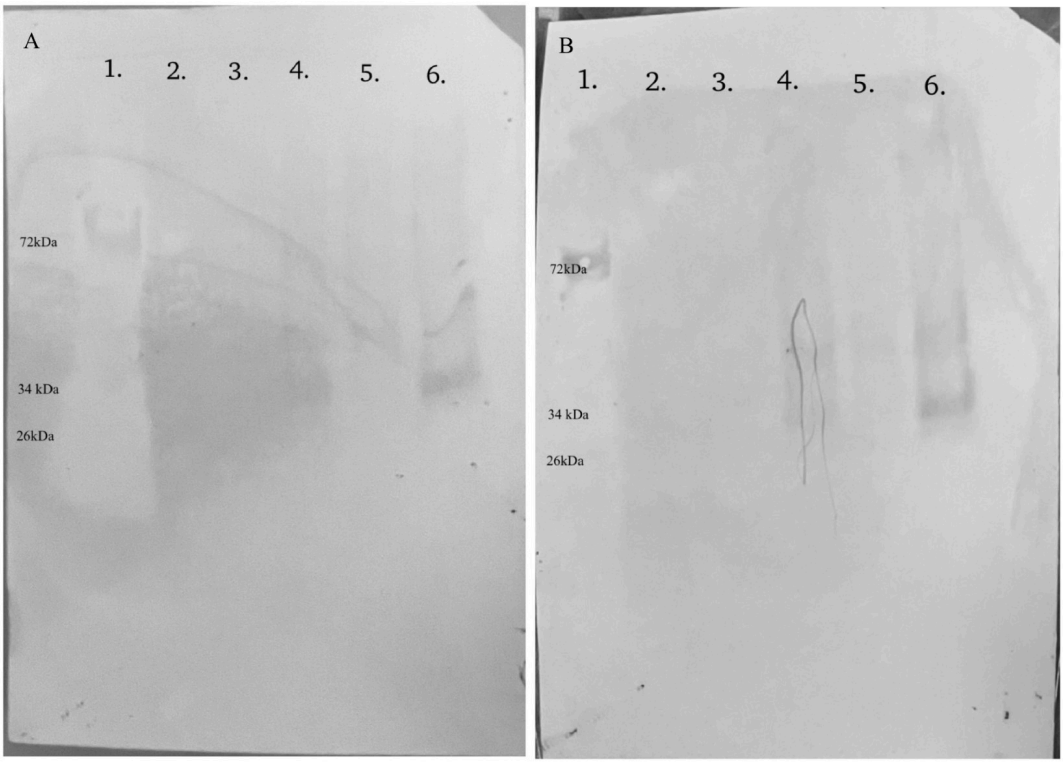

Figure 12. (A) Trial 3 Immunoblot 1 (1: marker; 2: Media; 3: Methanol; 4: Soybeans; 5: Solaray®; 6: MCF-7) (B)Trial 3 Immunoblot 2 (1: marker; 2: Media; 3: Methanol; 4: Soybeans; 5: Solaray®; 6: MCF-7)

In the final immunoblot experiment, the secondary antibody used was Goat Anti-Mouse IgG (H+L) Alkaline Phosphatase Conjugated (Invitrogen 31320) similar to the first experiment. In addition to the normal treatments previously experimented with, serum-starved MCF-7 breast cancer cells were also tested. These cells were forced into apoptosis to confirm the functionality of the Caspase-3 primary antibody. Figures 12a and 12b display the immunoblots from the final experiment and the appearance of bands at 43 kDa and 32 kDa in the soybean, Solaray®, and MCF-7 serum-starved treatment wells. There were no bands present in the control and methanol treatment wells suggesting there was not enough protein applied to the wells during gel electrophoresis. An alternate explanation could be that cell proliferation did occur in these wells but too little for PCNA to be detectable though immunoblot based on the cell dose assays. Bands at 36 kDa would have indicated that those treatments exhibited PCNA and proliferation but the bands that did appear were too high to be PCNA, it could not be determined what could have caused those bands. Treatments with bands at 34 kDa indicate the presence of intact Caspase-3, however, if the cells had gone through apoptosis Caspase-3 would have been cleaved. Cleaved Caspase-3 would have appeared at 19 kDa and none of the wells exhibited this, even though MCF-7 cells were used as a control for this biomarker. These immunoblots suggest that any reduction in treated cells was due to a reduction in proliferation rather than apoptosis occurring but which treatment was most effective was not able to be assessed due to the faintness of the bands and inconsistent protein concentrations.

Conclusion and Future Recommendations

Our results do not support our hypothesis that Phytoestrogen Solaray® would decrease ovarian cancer cell (OVCAR-3) proliferation. Soybeans and Phytoestrogen Solaray® seemed to show no consistent effects on cell growth, or viability in the cell line. The repeated experiments of a dose-response assay, western blot, and immunoblot showed no consistent results when performed. MCF-7 was used as a positive control in order to display activated Caspase-3 yet it was not active, suggesting human error when performing the protocol. According to the results of the immunoblot analysis, it indicates that the observed decrease in treated cells was likely attributed to decreased proliferation rather than apoptosis. The potential of the proliferation of OVCAR-3 cells will persist despite performing various experiments in order to decrease cell counts. This is due to the fact that some estrogens will bind to the ERα pathway which will initiate proliferation in the cells even though phytoestrogens have a higher affinity ERβ. However, determining the most effective treatment was hindered by the faintness of the bands and inconsistent protein concentrations.

Future studies should attempt to reestablish a model system where soybeans and Phytoestrogen Solaray® have an observational effect on OVCAR-3 cells, as no consistent observational effect was detected during the duration of the project. Future studies should run a High-Performance Liquid Chromatography (HPLC) analysis on the soybean and Phytoestrogen Solaray® extracts in order to determine the concentration of the isoflavones Genistein, Daidzein, and Glycitein. Conducting this analysis is crucial in determining the precise concentration of each isoflavone within each extract to help support the hypothesis that was made and to ensure the efficacy of the extracts in inducing the desired effects.

The use of an MTT assay is another essential component in order to minimize variations in cell seeding and also to ensure consistent experimental conditions in each of the wells that are going to be plated. The use of an MTT assay will ensure uniform cell numbers and accurate metabolic activity in each of the wells that contain OVCAR-3 cells. This is critical for interpreting the true effects of the extracts on cell viability and proliferation.

Cell culture medium components significantly influence experimental outcomes, particularly when studying the effects of estrogen-like compounds. Substituting phenol red with DCC serum will help mitigate unintended estrogen binding and minimize experimental confounders. This will enhance the specificity and accuracy of the observed cellular responses to the extract treatments.

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