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Analysis of the Anti-Cancer Effects of *Taraxacum officinale* Seed Extract

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Analysis of the Anti-Cancer Effects of *Taraxacum officinale* Seed Extract

A THESIS

APPROVED FOR THE DEPARTMENT OF BIOLOGY

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ABSTRACT OF THESIS

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ABSTRACT:

Taraxacum officinale, known as dandelion, has been used in traditional medicines for centuries. In recent years, researchers have started to investigate the potential anti-cancer effects of dandelion extracts. The purpose of this thesis was to determine the gaps in and expand upon the current research involving these extracts. It was determined that experimentation on the anti-cancer effects of extracts derived from the seeds of *T.officinale* was lacking in the current literature. Cancerous HeLa cells and non-cancerous human dermal fibroblasts (HDF) cells were treated with dandelion seed extract (DSE), and the effect was studied; focusing on the viability and migration of cells along with an analysis of the effects on gene activity- specifically on SFRP1, Axin2, and BMF- via transcriptomic analysis and western blot testing. BMF is involved in apoptosis. Axin2 and SFRP1 are involved in the Wnt Pathway. It was found that there was no significant difference between the effect the DSE treatment had on the viability of the HeLa cell versus on the HDF cells. The migration assay showed that there were significant differences between the cancerous and non-cancerous cells at certain concentrations. It

was shown, and later confirmed via western blots, that the DSE treatment affected the above genes in a way that suggested treatment caused a decrease in apoptotic activity and an increase in the activity of the Wnt Pathway in cancerous cells more than in non-cancerous cells.

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TABLE OF ABBREVIATIONS

Abbreviation	Definition
DWE	Dandelion whole extract
DFE	Dandelion flower extract
DLE	Dandelion leaf extract
DRE	Dandelion root extract
DSE	Dandelion seed extract
HDF	Human dermal fibroblasts
BMF	Bcl2 modifying factor
SFRP1	Secreted frizzled related protein 1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
ITIS	Integrated taxonomic system
ESCC	Esophageal squamous cell carcinoma
JSTOR	Journal storage
TNBC	Triple-negative breast cancer
TAM	Tumor-associated macrophage
NSCLC	Non-small-cell lung cancer
ERK	Extracellular signal-regulated kinase
JNK	c-Jun amino-terminal kinase
BCSC	Breast cancer stem cell
ROS	Reactive oxygen species
DSS	Dextran sodium sulfate
RNA	Ribonucleic acid
CCAT	Colon cancer associated transcript
CMML	Chronic myelomonocytic leukemia
EAF	Ethyl acetate
WF	Water fraction
UV	Ultraviolet
ATCC	American Type Culture Center
DMEM	Dulbecco's modified eagle medium
FBS	Fetal bovine serum
RB	Reagent blank
Con	Control
ANOVA	Analysis of variance
mRNA	Messenger RNA
DNA	Deoxyribonucleic acid
MPER	Mammalian protein extraction reagent
RPM	Rotations per minute
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
SB	Sample buffer
TBST	Tris-buffered saline solution with Tween-20
DMSO	Dimethyl sulfoxide
NAD	Nicotinamide adenine dinucleotide
NADH	NAD + Hydrogen

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
DOX	Doxorubicin
ETOP	Etopside
Bcl2	B-cell leukemia lymphoma 2 protein
LRP	Low-density lipoprotein receptor-related protein
CRD	Cysteine-rich domain
Bid	BH3 interacting-domain death agonist
Bax	Bcl2 associated x-protein
Apaf	Apoptotic peptidase activity factor
BH3	Bcl2 homology domain 3
UCO	University of Central Oklahoma

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In Vitro Anticancer Effects of *Taraxacum* Genus Extracts: A Review

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Abstract

Cancer is one of the leading causes of death across the globe, affecting millions of lives. Natural products derived from traditional medicines have been used to treat many illnesses including cancer. Taraxacum officinale, commonly known as dandelion, and other similar species have been a growing topic of interest for their potential anticancer effects. A review of the current literature showed that researchers have experimented with crude extracts from different organs of the dandelion plant, notably root, leaf, flower, and whole plant extracts, to show their effect on cancer cell lines. A comparison of these extracts' anticancer potential was conducted. Based on published literature, research has "room to grow" studying extracts from dandelion leaves, seeds, and flowers, in addition to numerous untested species. Several studies have conducted phytochemical analysis and assessed the cytotoxicity of both the crude extract and its individual fractions. Most studies have found that none of the isolated fractions exhibited the same level of potency as the crude extract. To unravel how these distinct components combine

to replicate the effects of traditional medicines, a synergistic research approach is required to identify the optimal combination of these fractions or bioactive molecules.

Introduction

Taraxacum officinale, more commonly known as the dandelion, can be traced back to glacial and interglacial times in Europe (Godwin 1956). It is believed that various species of the genus Taraxacum colonized the western hemisphere prior to the Gondwana supercontinent split about 180 million years ago (Richards 1973). It has been used in herbal Native American, Mexican (Sansores-España, Pech-Aguilar et al. 2022), Greek, Chinese medicine, and others (SharifiRad, Roberts et al. 2018) for centuries due to its anti-inflammatory and anti-oxidative properties (Yarnell and Abascal 2009). In the United States, dandelions are considered invasive weeds that serve no purpose. Dandelions are found throughout Oklahoma (Palmer 2007, 2022), and are the second earliest blooming plant in central Oklahoma (Osborn 2015). What makes these weeds so special and gives them the potential to aid in treating one of the most prolific diseases seen across the globe?

According to the Integrated Taxonomic Information System (ITIS), as of March 2023, there are 15 species of Taraxacum in addition to Taraxacum officinale (Brouillet 2023). Medicinal aspects of the genus Taraxacum have been a popular topic of academic studies for decades. Almost half of the reported biomedical research are from studies on Taraxacum officinale alone (Martinez, Poirrier et al. 2015).

Due to the versatility of uses these plants have offered to holistic and herbal medicine cultures, scientists have been attempting to validate these uses for more clinical settings. In particular, the antimicrobial effects of the Taraxacum genus have been

thoroughly investigated. Researchers found that the plants were effective in inducing growth arrest, or “halo zones” in agar plates of multiple bacteria. Notably, different species of Taraxacum plants were found to create halo zones against Klebsiella pneumoniae (Shahidi Bonjar, Aghighi et al. 2004), Staphylococcus aureus (Demin 2010), and Bacillus subtilis (Tahir, Nazir et al. 2017), all of which are incredibly common in the environment and are known to cause many different aliments.

In more recent years, multiple studies have examined the possible anticancer effects of Taraxacum plants to fight against more serious illnesses. There has been a spike in researchers running experiments to examine the possible anticancer effects of *Taraxacum officinale* and related Taraxacum species. Cancer is the second leading cause of death in the United States. In 2021, approximately 1.9 million new cancer cases were diagnosed, and 608,570 cancer-caused deaths occurred (Siegel, Miller et al. 2022). As the number of cancer diagnoses increases, the number of new forms of cancer therapy increases. However, there continues to be a high rate of death associated with these cancers. The need for new, innovative, less expensive cancer therapies having fewer harsh side effects is ever growing.

While many studies have identified compounds present in Taraxacum, there is surprisingly less research on crude extracts or compound mixtures to determine if such combinations will provide similar effects to those reported with traditional medicines (Martinez, Poirrier et al. 2015, Scaria, Sood et al. 2020). The purpose of this review is to focus on dandelion crude extract research reported in the literature, with specific emphasis on in vitro cancer cell culture studies. These studies include isolated plant parts or mixtures, or the whole plant. During active growth, dandelions continuously

demonstrate leaves and roots; less often, other parts related to reproduction (e.g., flowers, seeds, petals, bracts, seed heads) are present (Vijverberg, Welten et al. 2021) (Table 1). Many of the studies reported here do not adequately describe which parts are included in the extracts that were tested, nor which species was extracted, leaving room for interpretation of results.

Table 1. Summary of dandelion crude extracts tested in experimental models.

Extract Type	Species Extracted	Experimental model*	Cell lines	Refs
Whole Plant Extract	*T. officinale*	Breast cancer stem cells	Primary cell culture	(Trinh, Doan-Phuong Dang et al. 2016)
		Brest cancer cells	MCF-7	(Rawa'a, Dhia et al. 2018)
		Normal liver cells	WRL-68	(Rawa'a, Dhia et al. 2018)
		Pediatric cancer cells (18)	RAMOS, MV4-11, etc	(Menke, Schwermer et al. 2018)
		Normal human fibroblasts	NHDF-C	(Menke, Schwermer et al. 2018)
	T. formosanum	Bronchial epithelial cells	BEAS-2B	(Chien, Chang et al. 2018)
		Lung adenocarcinoma cells (2)	CL1-0 CL1-5	(Chien, Chang et al. 2018)
		Breast cancer cell lines (3)	MDA-MB-231 ZR75-1 MCF-7	(Lin, Chen et al. 2022)
	T. mongolicum	Breast cancer cell lines (3)	MDA-MB-231 ZR75-1 MCF-7	(Lin, Chen et al. 2022)
		Monocytic leukemia cells	U937	(Deng, Jiao et al. 2021)
		Breast cancer cells (2)	MDA-MB-231 MDA-MB-468	(Deng, Jiao et al. 2021)
		Breast cancer cells (2)	MDA-MB-231 MCF-7	(Li, He et al. 2017)
		Embryonic kidney cells	HEK293	(Li, He et al. 2017)

		Breast cancer cells (2)	MDA-MB-231 MDA-MB-468	(Wang, Hao et al. 2022)
		Normal mammary epithelial cells	MCF-10A	(Wang, Hao et al. 2022)
Unknown source	*Taraxacum sp*.	Lung adenocarcinoma cells	A549	(Man, Wu et al. 2022)
Root Extract	*T. officinale*	Breast cancer cells	MCF-7/AZ	(Sigstedt, Hooten et al. 2008)
		Prostate cancer cells	LNCaP C4-2B	(Sigstedt, Hooten et al. 2008)
		Normal colon mucosal epithelial cells	NCM460	(Ding and Wen 2018)
		Prostate Cancer cells (2)	DU-145 PC-3	(Nguyen, Mehaidli et al. 2019)
		Colonic epithelial cells	FHC	(Nguyen, Mehaidli et al. 2019)
		Colon cancer cells (2)	HT-29 HCT116	(Ovadje, Ammar et al. 2016)
		Colonic epithelial cells	NCM460	(Ovadje, Ammar et al. 2016)
	Taraxacum sp.	Esophageal SCC (4)	KYSE450 NEC Eca109 EC9706	(Duan, Pan et al. 2021)
		Normal esophageal cells	NE3	(Duan, Pan et al. 2021)
		Cancer cells (3)	HepG2 MCF7 GCT116	(Rehman, Hamayun et al. 2017)
		Normal cells	HS27	(Rehman, Hamayun et al. 2017)
		Gastric cancer cells (2)	SGC7901 BGC823	(Zhu, Zhao et al. 2017)
		Normal gastric epithelial cells	GES-1	(Zhu, Zhao et al. 2017)

		Melanoma cells (2)	A375 G361	(Chatterjee, Ovadje et al. 2011)
		Normal human fibroblasts	Primary	(Chatterjee, Ovadje et al. 2011)
		T-cell leukemia (2)	Jurkat E6-1 dnFADD Jurkat	(Ovadje, Chatterjee et al. 2011, Ovadje, Hamm et al. 2012)
		Peripheral blood mononuclear cells	Primary	(Ovadje, Chatterjee et al. 2011, Ovadje, Hamm et al. 2012)
		Chronic myelomonocytic leukemia cells (3)	MV-4-11 HL-60 U-937	(Ovadje, Hamm et al. 2012)
		Pancreatic cancer cells (2)	BxPC-3 PANC-1	(Ovadje, Chochkeh et al. 2012)
		Normal human fibroblasts	Primary	(Ovadje, Chochkeh et al. 2012)
Leaf Extract	T. officinale	Breast cancer cells	MCF-7/AZ	(Sigstedt, Hooten et al. 2008)
		Prostate cancer cells	LNCaP C4-2B	(Sigstedt, Hooten et al. 2008)
	Taraxacum sp.	Colon cancer cells	HT-29	(Xue, Zhang et al. 2017)
Flower Extract	*T. officinale*	Breast cancer cells	MCF-7/AZ	(Sigstedt, Hooten et al. 2008)
		Prostate cancer cells	LNCaP C4-2B	(Sigstedt, Hooten et al. 2008)
		Colorectal cancer cells	Caco-2	(Hu and Kitts 2003)
Seed Extract	*T. officinale*	Hypopharyngeal cancer cells	FaDu	(Milovanovic, Grzegorczyk et al. 2022)

		Cervical Cancer cells	HeLa	(Milovanovic, Grzegorczyk et al. 2022)
		Kidney epithelial cells	Vero	(Milovanovic, Grzegorczyk et al. 2022)
		Normal fibroblasts	CCD-1059Sk	(Milovanovic, Grzegorczyk et al. 2022)
	Taraxacum sp.	Esophageal SCC cells (5)	KYSE450 Ecal109 NEC EC9706 TE-13	(Li, Deng et al. 2022)
*Number in parentheses indicates number of cell lines tested				

In this work, we review the current state of knowledge regarding the anticancer effects of Taraxacum officinale and related species. Because the dandelion is a complex plant with a diverse biochemical composition, coupled with variations in the parts utilized across different studies, we decided to structure the review into sections by the part of the plant used (whole plant extract, roots, roots, leaves, flowers, and seeds). We then discuss the overall knowledge based on analysis of the data and highlight potential areas for future research.

Methods

We searched databases available online and written in English through the University of Central Oklahoma, including Google Scholar, JSTOR, ProQuest, PubMed, Science Direct, and Web of Science using keywords such as “taraxacum” or “dandelion” with “crude extract”, “in vitro” “phytochemistry”, “pharmacology” “cancer cell”. Each abstract was read to determine if crude extracts of Taraxacum were used, and what kind of in vitro cells/experiments were conducted. From this evaluation, the papers collected were read in their entirety for further preparation.

Dandelion Whole Plant Extracts

Multiple studies using extracts from the whole dandelion plant (dandelion whole extract, or DWE) have been reported. DWE had a negative effect on triple-negative breast cancer (TNBC) migration, proliferation, and its ability to invade tumor-associated macrophages (TAMs) (Deng, Jiao et al. 2021). When tested on non-small-cell lung cancer (NSCLC), DWE exhibited antioxidant effects as well as anticancer effects. DWE was unable to decrease cancer cell proliferation in a significant manner, but it was able to decrease the number of colonies formed by the cancer. It was also found that DWE exhibited inhibitory effects on the migration of cancer cells. DWE treatment decreased phosphorylation of ERK1/2 but did not have effects on p38 and JNK1/2 (Chien, Chang et al. 2018). In another study using breast cancer stem cells (BCSC), DWE was shown to inhibit BCSC proliferation by inducing apoptosis. DWE also increased the ROS in cancer stem cells (Trinh, Doan-Phuong Dang et al. 2016). While these studies reported effects only on diseased cells, another study compared DWE effects on cancer vs. non-cancerous cells, using MCF-7 (human breast cancer) cells against WRL-68 (normal human hepatic) cells. The study demonstrated that DWE significantly reduced cell viability of the cancerous cells but not the non-cancerous cells (Rawa'a, Dhia et al. 2018). Another study tested a panel of 18 cancer cell lines against a normal human fibroblast line and found DWE was more potent against the cancer cells than normal cells (Menke, Schwermer et al. 2018).

One study using T. mongolicum and T. formosanum aqueous extracts tested against 3 breast cancer cell lines showed mixed results on the cell lines; both extracts reduced cell migration and colony formation, but T. mongolicum was more cytotoxic to

the tested cell lines (Lin, Chen et al. 2022). A similar study found T. mongolicum DWE extract inhibited triple-negative breast cancer cell viability and induced apoptosis (Li, He et al. 2017). A follow-up study using a multi-omics approach showed the effects were exerted mainly by seven compounds including luteolin, and through interference with lipid metabolism (including phospholipid and fatty acid metabolism) (Wang, Hao et al. 2022).

A crude extract from an unknown dandelion species and plant part was tested against A549 lung adenocarcinoma cells, where it was shown to be cytotoxic in a dose-dependent manner (Man, Wu et al. 2022). Further investigation into the metabolomic profile showed deficiencies in purine metabolism, but also glycerophospholipid metabolism, which was also reported in (Wang, Hao et al. 2022). These and other noted metabolic changes suggested DWE affected malignant proliferation, membrane stability/structure, and cells' ability to adhere to their extracellular matrix, all providing the stimulus for apoptosis.

While these studies offer substantial evidence supporting the potential therapeutic benefits of DWE, the absence of detailed information regarding specific plant parts included in the DWE description introduces some ambiguity when interpreting or comparing results with other studies. Additionally, the extraction methods varied from cold aqueous, hot aqueous, or ethanol to ethyl acetate processes (Lin, Chen et al. 2022; Deng, Jiao et al. 2021; Chien, Chang et al. 2018). This variability suggests that the substances extracted for testing may differ, potentially yielding diverse results.

Dandelion Root Extracts

One part of the dandelion that is consistently present year-round is the root, and consequently much of the published in vitro cancer studies focused on dandelion root extracts (DRE). Root aqueous extracts reduced viability but not cell growth of LNCaP C4-2B prostate cancer cells and MCF-7/AZ breast cancer cells. ERK phosphorylation was unaffected in either of the cell lines. DRE treatment blocked in vitro collagen invasion of MCF-7/AZ but not LNCaP C4-2B (Sigstedt, Hooten et al. 2008). Another study showed aqueous DRE was effective against esophageal squamous cell carcinoma (ESCC) cell growth, proliferation, migration, and invasion, but was less effective against normal esophageal cells. DRE inhibited in vivo tumorigenesis and induced apoptosis (Duan, Pan et al. 2021).

Another DRE study combined in vitro testing with an in vivo mouse model. Aqueous DRE increased cell viability and prevented apoptosis of NCM460 colonocytes induced by dextran sodium sulfate (DSS), correlated with reduced ROS production. Using female C57BL/6 mice as a model for humans, DRE inhibited DSS-induced ulcerative colitis and reduced inflammation and oxidative stress in the colon of the mice (Ding and Wen 2018).

A series of studies with DRE compared cancer cell effects against non-cancerous cells, to provide further support for future clinical studies. One study compared DRE effects on gastric cancer versus normal gastric epithelial cells (Zhu, Zhao et al. 2017). DRE inhibited cancerous but not normal gastric cells, in part by targeting a long-noncoding RNA, CCAT1. Another study showed DRE (aqueous extract)- treated A375 melanoma cells exhibited a decrease in cell viability, while having no viability reduction

in normal human fibroblasts. DRE treatment induced caspase-8 dependent apoptosis in the melanoma cells, correlated with increased mitochondrial ROS production. Other apoptotic markers were observed, including dissipation of the mitochondria membrane potential. Higher DRE doses were necessary to decrease viability in another melanoma cell line (G361) compared to the A375 cell line (Chatterjee, Ovadje et al. 2011). Another study compared aqueous DRE with ethanolic extract of lemongrass, using both in vitro and xenograft in vivo testing. DRE induced caspasedependent apoptosis in two prostate cancer cell lines, but not normal colonic epithelial cells. Using immunocompromised CD1 nu/nu mice as an in vivo model, DRE was able to reduce tumor weight and volume (Nguyen, Mehaidli et al. 2019). When aqueous DRE was tested against human leukemia (Jurkat) cells, the higher the concentration of DRE used to treat the cells, the higher the amount of apoptosis was exhibited by the cells. DRE was able to reduce the cell viability of the leukemia cells by 60%. This apoptosis was shown to be caused by the activation of caspase 8, which then activated caspase 3, resulting in apoptosis. To ensure that the DRE was not damaging non-cancerous cells, peripheral blood mononuclear cells were treated with DRE and there were no significant damage and changes reported after treatment (Ovadje, Chatterjee et al. 2011). Another human leukemia cell line derived from chronic myelomonocytic leukemia (CMML) demonstrated 60% viability decrease. The remaining viable 40% were treated with a second round of aqueous DRE, which induced apoptosis through the extrinsic pathway, damaged the mitochondrial membrane, and induced autophagy. Normal peripheral blood monocytes under the same treatment regimen were unaffected (Ovadje, Hamm et al. 2012). In a similar fashion, aqueous DRE was tested on pancreatic cancer cells (BxPC-3 and PANC-1) versus non-cancerous pancreatic cells.

DRE was shown to induce apoptosis in the BxPC-3 cells at 48 hours and PANC-1 cells at 24 hours. Multiple low-concentration doses were more effective inducing apoptosis than one large concentration dose. As with the Jurkat and CMML results, apoptosis occurred through the extrinsic pathway and autophagy was induced. These treatments were nontoxic to normal human fibroblasts (Ovadje, Chochkeh et al. 2012). More recently, aqueous DRE was tested against colon cancer cells (HT-29 and HCT166) and decreased cell viability in both cell lines by 50% yet did not decrease the cell viability of non-cancerous colon epithelial cells (NCM460). When a migration scratch assay was performed, DRE was successful in inhibiting cell migration of HT-29 and HCT166 cell lines while failing to inhibit the NCM460 cell line. DRE increased ROS in the cancerous HT-29 cells but not the non-cancerous colorectal cells. Multiple pathways of apoptosis were induced in the colorectal cancer cells. (Ovadje, Ammar et al. 2016). Because many of the DRE studies compared cancerous to non-cancerous cells, this in vitro evidence suggests that future studies in vivo are warranted.

Collectively, DRE has been tested on more cell types than other plant parts. The results indicate that DRE selectively inhibits cancerous cells, compared to normal cells. Similar to DWE, various preparation methods were used, and unnamed species extracted, making it challenging for future studies to reproduce reported results. The intricate mechanisms behind cell-type selectivity and potential off-target effects remain to be determined. Nevertheless, these findings suggest a promising avenue for future in vitro, in vivo, and clinical studies to determine the therapeutic potential of DRE.

Dandelion Leaf Extracts

Numerous studies have investigated the antioxidant potential of dandelion leaf extract (DLE) to protect against ROS damage in various cell and animal models; however, the published data on DLE effects on cancer cells is limited. Aqueous DLE was able to decrease the viability of MCF-7/AZ breast cancer cells and LNCaP C4-2B prostate cancer cells by 50%; cell growth of the breast cancer cell line was reduced by 40%. The DLE treatment resulted in a decrease of ERK activity, but not a decrease in the levels of ERK in the breast cancer cell line. DLE treatment did not influence the prostate cancer's cell growth. Finally, it was discovered that DLE was unable to inhibit the in vitro collagen matrix invasion of MCF-7/AZ cells. DLE was able to block the invasion of LNCaP C4-2B cells (Sigstedt, Hooten et al. 2008).

Another study compared the amount of phenolics and flavonoids in extracts taken from the root, flower, stem, and leaves of the dandelion (Xue, Zhang et al. 2017). 50% ethanolic DLE contained a higher phenolic content than all the other extracts while extracts from the leaves and the flowers were found to have the highest flavonoid content than the other extracts. This study also compared the antioxidant activity between dandelion root, flower, stem, and leaf extracts. The high phenolic content of DLE was correlated with higher antioxidant activity over the other extracts. Further DLE experiments concluded that DLE had a suppressive effect on the ROS production of the HT-29 cells (human colonic epithelial cells). Finally, antiinflammatory activity testing was performed using HT-29 cells. DLE inhibited activation of p65 (an NF-kappa B signaling molecule) and inhibited inflammatory signaling molecules (Xue, Zhang et al. 2017).

Dandelion Flower Extracts

Few studies have been conducted using Taraxacum officinale flowers. In one study, flower extracts were tested for anticancer effects on human colon colorectal adenocarcinoma (Caco-2) cells, using ethyl acetate (EAF) and a water fraction (WF). The DFEs were subjected to antioxidant testing and it was observed that DFE suppressed the formation of conjugated dienes and led to prolonged lag phase durations and lower rates of propagation. It was found that a high ROS concentration of EAF DFE prevented the formation of conjugated dienes and the negative charge of hLDL. The WF DFE showed no antioxidant activity. Both EAF and WF DFEs were cytotoxic to Caco-2 cells at a concentration of 0.1 mg/mL. HPLC profile showed the presence compounds including luteolin and luteolin-7 glucoside, which were shown to be more cytotoxic than EAF and WF (Hu and Kitts 2003).

Aqueous DFE, among other extracts, was tested against MCF-7/AZ breast cancer cells and LNCaP C4-2B prostate cancer cells to observe the anticancer effects against these cell lines. The viability of the breast cancer cells was unaffected by DFE treatment. However, prostate cell viability decreased significantly in the presence of DFE. Researchers ran assays to see if DFE could inhibit ERK, and therefore stop cell growth, in both cancer cell lines. It was concluded that DFE treatment did not affect ERK and cell growth in either of the cell lines. Both cancer lines were able to invade an in vitro collagen invasion assay. Through additional assays it was shown that DFE treatment could not block this invasion in MCF-7/AZ cells nor in the LNCaP C4-2B cells (Sigstedt, Hooten et al. 2008)

Dandelion Seed Extracts

Dandelion seeds and fruits are similar; the fruit functions akin to a seed coat and is difficult to separate from the seed (Dr. Jenna Messick, UCO, personal communication). While some dandelion fruit extract (DFE) antioxidant studies have been published, no published cancer reports using dandelion fruit extracts were identified. Two publications about the medicinal properties of dandelion seed extract (DSE) were recently published. DSE was able to reduce the survival and proliferation rates of esophageal squamous cell carcinoma (ESCC) cells. Additionally, DSE induced apoptosis and suppressed migration, invasion, and angiogenesis (Li, Deng et al. 2022). A second study tested DSE against hypopharyngeal cancer (FaDu), cervical adenocarcinoma (HeLa), and colon cancer (RKO) cells versus normal kidney cells and skin fibroblasts. When all cell types were tested for cytotoxicity, the extract showed selectivity for the hypopharyngeal and colon cancer cells compared to the normal cells, while HeLa cells were resistant to the DSE (Milovanovic, Grzegorczyk et al. 2022).

Dandelien extract effects when combined with other plant extracts

An additional publication described research using extracts that contain a mixture of mushrooms and plants, including dandelion. Specifically, the mixture included Coix seed, Lentinula edodes (shiitake mushroom), Asparagus officinalis L., Houttuynia cordata (chameleon plant), Dandelion, and Grifola frondosa (hen of the wood mushroom) (Chen, Yue et al. 2021).

Discussion

Dandelion has a long history of use as a medical herb. It has been shown to have antioxidant and anti-inflammatory benefits. Many of the above publications are from

recent years, especially those that work with dandelion seed extract. This research is still in its infancy and needs to be expanded upon in future experiments. Since there are so few publications on this topic pertaining to each part of the dandelion, more experiments will need to be conducted with each extract before the research can move towards clinical use.

Because research teams used different types of cancer cells for their experiments it is difficult to make direct comparisons between the different types of extracts. However, there are clear trends that these extracts do, in fact, exhibit anticancer effects. Many of the researchers ran similar assays, including proliferation, viability, and migration assays. There is evidence that membrane stability is compromised through lipid metabolism effects. In nearly every case, no matter which extract was used and no matter what cancer cell lines were used, the cancer cells were inhibited by the extract treatment. This indicates a trend that dandelion extracts are showing signs of having anticancer properties that should have limited cytotoxicity to the noncancerous cell environment.

In more recent years, researchers have begun fractionating dandelion extracts to find their biocomponents, in hopes of isolating purified anticancer chemicals. These publications evaluated the total flavonoids of an extract (He, Han et al. 2011, Kang, Miao et al. 2021), dandelion polysaccharides (Ren, Yang et al. 2021), and specific components, such as inulin fructan (Zhang, Song et al. 2021), taraxasterol (Ovadje, Ammar et al. 2016), and luteolin (Tsai, Tsai et al. 2021). The phytochemical analysis of dandelion roots, leaves, and flowers has been published extensively. Common components found in all three include caffeic acid, chlorogenic acid (Hu and Kitts 2003, GonzálezCastejón, Visioli et al. 2012, Xue, Zhang et al. 2017), syringic acid, ferulic acid, and chicoric acid

(González-Castejón, Visioli et al. 2012, Xue, Zhang et al. 2017) (Table 2). While there is one publication detailing the phytochemical components of dandelion fruit (Lis, Jedrejek et al. 2020) there are no recent publications showing the phytochemical components of dandelion seeds.

Table 2. Phytochemical components of extracted dandelion plant parts.
Data are from references: (Hu and Kitts 2003), (González-Castejón, Visioli et al. 2012), (Xue, Zhang et al. 2017).

Biocomponent	**DWE**	**DRE**	**DLE**	**DFE**
10,15-octadecadienoic acid	x			
11,13-dihydro-taraxinic acid β-glucopyranoside		x	x	
11β,13-dihydrolactucin		x		
4-caffeoylquinic acid	x	x		
4-coumaric acid			x	x
4-hydroxybenzoic acid	x			
9,10,11-trihydroxy-(12Z)-octadecanienoic acid	x			
9,10,11-trihydroxy-9,11-octadecadienoic acid	x			
Acylated γ-butyrolactone glycoside		x		
Aesculin			x	
Ainslioside		x		
Apigenin 7-*O*-glucoside			x	
Apigenin				x
Arnidiol		x	x	
Caffeic acid	x	x	x	x
Caffeoyl hexoside	x			
Caffeoyl-D-glucose	x			
Caftaric acid	x			
Chicoric acid	x	x	x	x
Chlorogenic acid	x	x	x	x
Chrysoeriol			x	
Cichoriin			x	
Cis-caftaric acid	x			
Esculetin	x	x		
Faradiol		x		
Ferulic acid	x	x	x	x
Gallic acid			x	
Hesperidin	x			
Hydroxy-10,12,15-Octadecatrienoic acid	x			
Hydroxycinnamic acid derivative	x			
Isorhamnetin	x			x

Ixerin D		x		
Lupeol		x		
Luteolin	x			x
Luteolin 7-diglucoside			x	
Luteolin 7-glucoside	x		x	x
Luteolin hexoside	x			
Luteolin-7-O-rutinoside	x		x	
Monocaffeoyltartaric acid		x	x	
Picrasinoside F.	x			
Protocatechuic acid		x		
Quercetin	x		x	x
Quercetin-7-O-glucoside			x	
Rutin			x	
Scopoletin		x		
Stigmasterol		x		
Syringic acid	x	x	x	x
Taraxacolide-O-β-glucopyranoside		x		
Taraxacoside		x		
Taraxasterol		x		
Taraxinic acid β-glucopyranoside		x	x	
Tetrahydroridentin B		x		
Trans-cinnamic acid			x	x
Umbelliferone		x		
Vanillic acid	x	x	x	
α-amyrin		x		
β-amyrin		x	x	
β-sitosterol		x	x	
β-sitosterol-β-D-glucopyranoside		x		
ρ-coumaric acid	x	x		
ρ-hydroxybenzoic acid		x	x	
ρ-hydroxyphenylacetic acid		x		

Cancer is not the only aliment that dandelion extracts have been shown to combat. A China based research group from the South-Central University for Nationalities used an ethyl acetate extract of dandelion and observed its anti-asthma effects (Sarı and Keçeci 2019). The Chinese Academy of Sciences published work showing the anti-influenza potential of dandelion (Zhao, Liu et al. 2020). A Canadian team showed that dandelion extracts could be used to protect human cells from UV radiation (Yang and Li 2015). In

2022, researchers from King Saud University used DSE to treat mice with hypercholesterolemia and determine the effect (El-Nagar, Al-Dahmash et al. 2022).

Much work has been done to characterize dandelion extracts, their chemicals, and effects in vitro and in vivo. However, many of these studies do not identify the species tested. Reported methods of extraction differ, leaving it difficult to compare one study to another. Studies often use different cell types for testing, making comparisons difficult. More clinical studies are needed (Li, Chen et al. 2022), but current knowledge should be fine-tuned before proceeding (Sharifi-Rad, Roberts et al. 2018), in order to compare results to traditional medicine (Martinez, Poirrier et al. 2015, Scaria, Sood et al. 2020). Only a few of the known dandelion species have been tested; untested species may have anticancer potential (Li, Chen et al. 2022). Future work with big data set analysis of phytochemical mixtures combined with observed health benefits and/or “symptomics” should begin to tease out specific benefits of dandelion constituents for future therapies.

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Master's Thesis

Justification

Many of the publications in the above manuscript are from recent years, especially those that work with dandelion seed extracts. This research is still in its infancy and needs to be expanded upon in future experiments before the research can move towards clinical use. Our team planned to focus on dandelion seed extract (DSE) testing as it is one of the least studied parts of the dandelion plant.

The Biology Department at the University of Central Oklahoma had been conducting this type of research with dandelion whole extract (DWE) but switched to focus on DSE in 2020/2021. The goal of this team had been to fractionate DSE and study its effects on cancerous and non-cancerous cells to fill the gap in this research. I aimed to further that work and chose to use DSE instead of other extracts because the publications pertaining to DSE are few and recent.

Following the publication of the literature review manuscript (above), I conducted viability and migration assays on cancerous and non-cancerous cells using DSE treatment at different concentrations. I then analyzed transcriptomics data set of the effect this DSE treatment has on these cells' proteins, choosing a few key genes for more intensive study. Finally, I used western blotting to confirm the effects on the specific proteins chosen.

Hypothesis/Objective of Study

The purpose of this research is to observe the anti-cancer potential of dandelion seed extract, specifically on cancerous HeLa cells versus non-cancerous human dermal fibroblasts (HDF) and determine the effect DSE treatment will have on these cells' viability and migration. The purpose is also to look at the effect DSE treatment has on

three specific genes- Axin2, BMF and SFRP1- in each cell type using transcriptomic data analysis and western blotting. This study will add to the research already being done on dandelions and their anti-cancer effects. We hypothesize that dandelion seed extract will be able to significantly decrease cell viability and migration of the HeLa cells compared to the HDF cells.

Materials and Methods

Cell Culture

HDF cells (cell lines: 00703 and 01035, purchased from LifeLine Cell Technology) and HeLa cells (purchased from ATCC) were cultured under the same conditions. Each were grown in T75 flasks in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS (fetal bovine serum). The flasks were incubated under standard conditions (37° C, 5% CO_2 and 90% humidity) until they reached 90-95% confluency. The use of these cells in these studies was approved by the UCO Institutional Review Board (IRB#08077).

Dandelion Seed Extract Preparation

The DSE needed for our experiments was provided by Dr. Amanda Waters of the Chemistry Department at the University of Central Oklahoma. *Taraxacum officinale* seeds were purchased from Amazon.com. The seeds were extracted with reagent alcohol that was approximately 90% ethanol, 5% methanol and 5% isopropanol [1]. This reagent mixture is a standard method to prepare a plant extract of this nature and was performed by Dr. Waters and her team.

Dandelion Seed Extract Treatment

Once the cells reached 90-95% confluency, some were subcultured into a 96-well plate with 20,000 cells per well- a number chosen through an analysis of current research suggesting between 17,000-35,000 cells be plated per well in a 96-well plate for 100% confluency [2]- being added to each well. The cells were incubated under standard conditions for 24 hours before DSE treatment. Different concentrations of dandelion seed extracts were added to each row of cells and 3 columns of cells were tested at a time with an extra column containing only reagents to serve as a reagent blank. The cells in the first row (Row A) of the plate, during each experiment, were left untreated by the dandelion extract and served as the negative control for the experiment. Figures 1 and 2 show the layout of treatments on the 96-well plates for each iteration of the experiment. The plate was returned to incubation in standard conditions after treatment.

Other cells were subcultured into a 48-well plate with 30,000 per well- manufacturer's instructions indicate 30,000 cells to be the approximate seeding number to achieve 100% confluency after 24 hours. [3] Three columns of HeLa cells and three columns of HDF cells were subcultured. The cells were incubated under standard conditions for 24 hours before DSE treatment. Different concentrations of dandelion seed extracts were added to each row of cells. The cells in the first row (Row A) of the plate were left untreated by the dandelion extract and served as the negative control for the experiment. Figure 3 show the layout of treatments on the 48-well plates. The plate was returned to incubation in standard conditions after treatment.

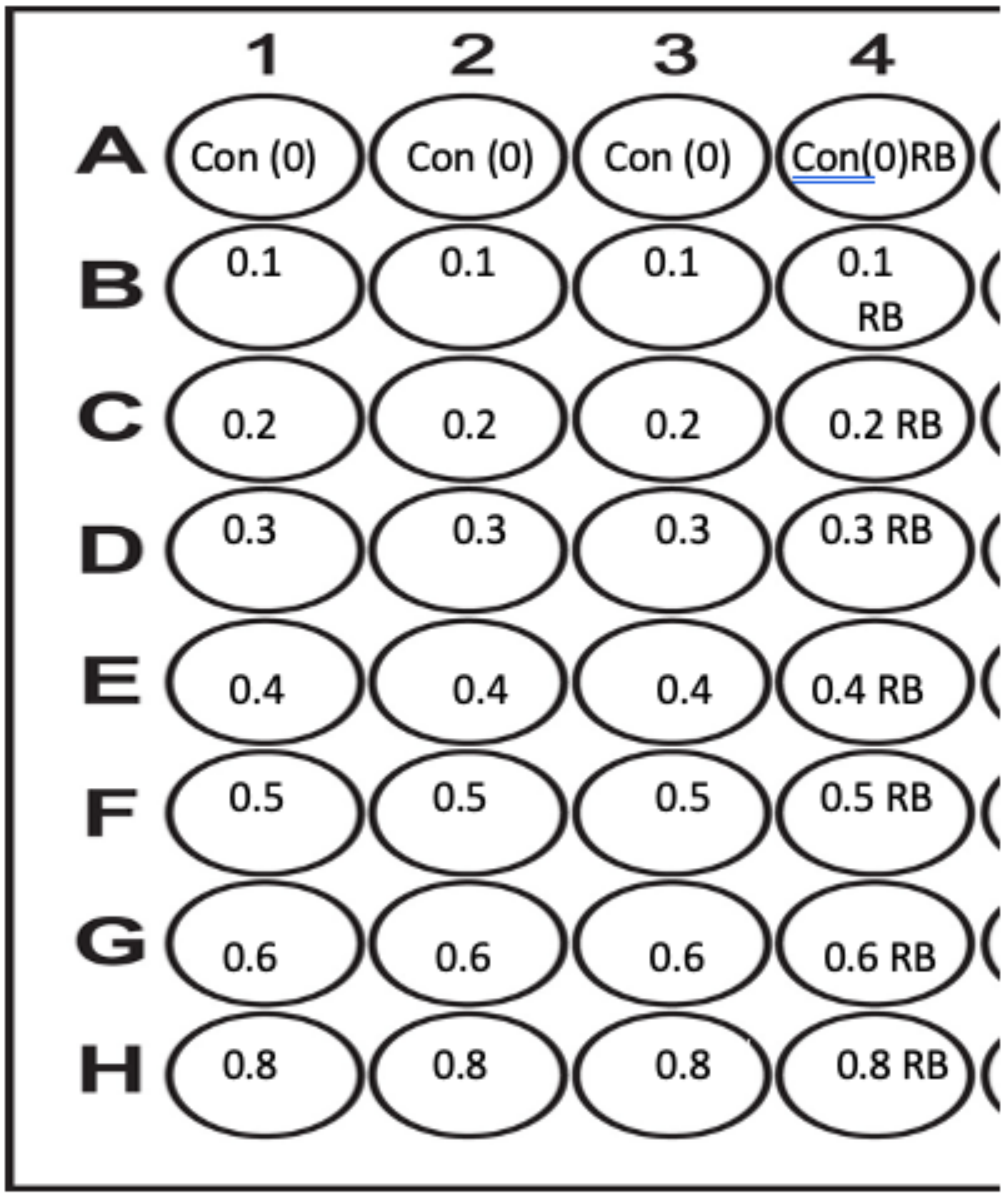


Figure 1*: A depiction of how the cells were treated with different concentrations of DSE in a 96-well plate in the first run of the viability assay. All wells that were not reagent blanks had 20,000 cells of either type subcultured onto them before treatment. Con=control, RB=reagent blank. All concentrations listed are in mg/ml.*

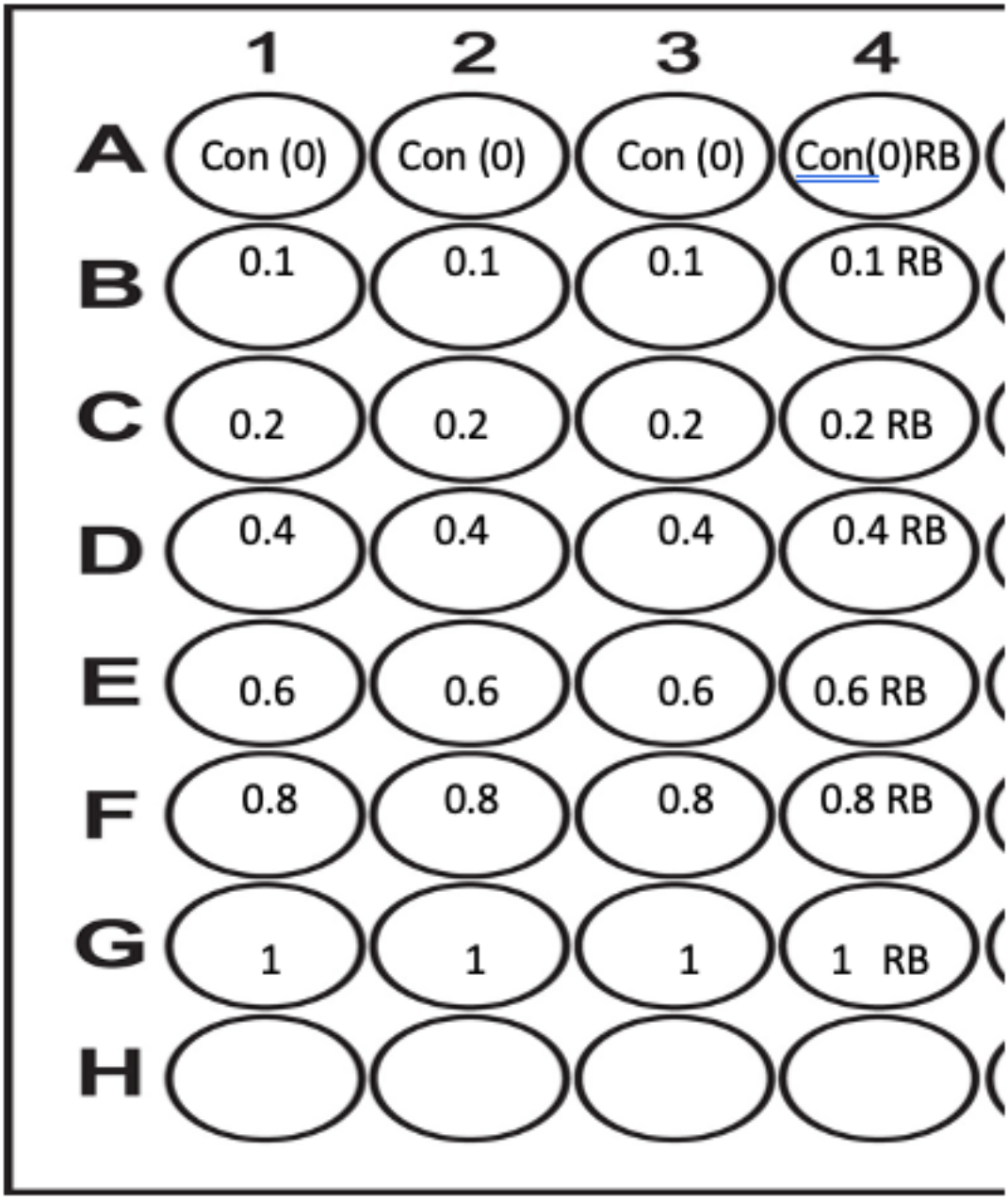


Figure 2: *A depiction of how the cells were treated with different concentrations of DSE in a 96-well plate in the second run of the viability assay. All wells that were not reagent blanks had 20,000 cells of either type subcultured onto them before treatment. Con=control, RB=reagent blank. All concentrations listed are in mg/ml.*

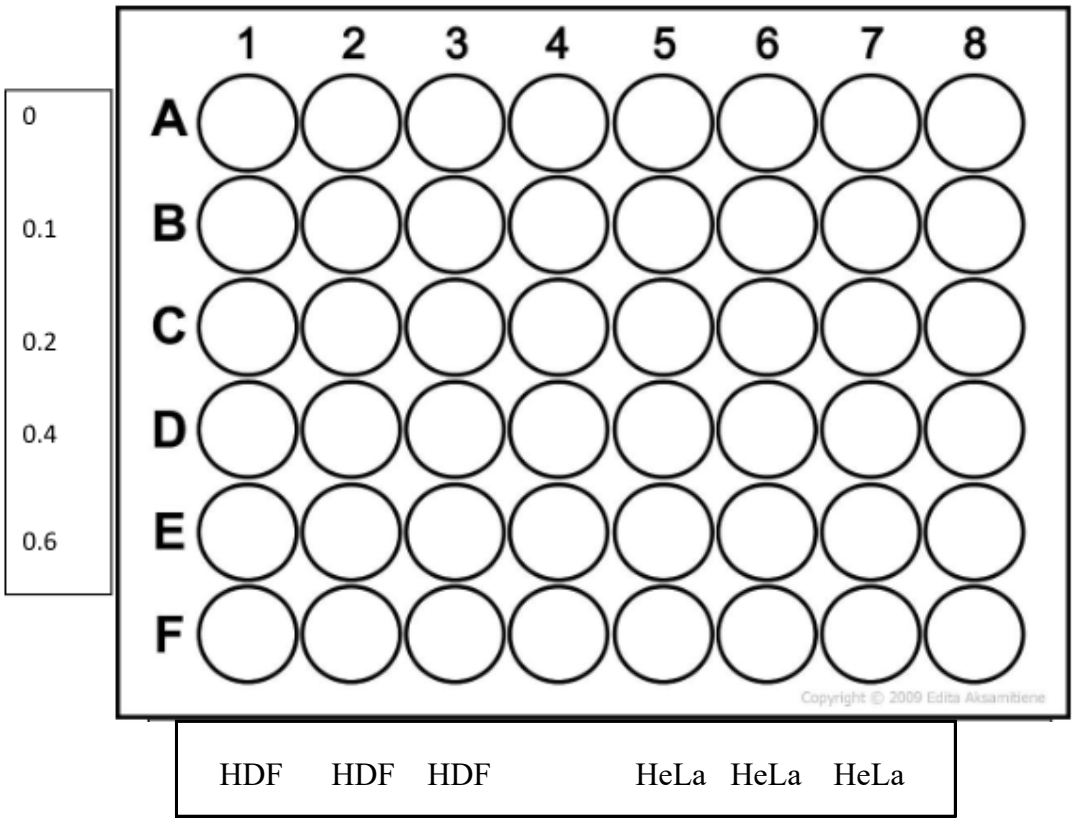


Figure 3: *A depiction of how the cells were treated with different concentrations of DSE in a 48-well plate. All concentrations listed are in mg/ml.*

Presto Blue HS Cell Viability Assay

After exactly 24 hours of incubation time after treatment, we incubated the cells in the 96-well plates in the presence of 10uL (per well) of Presto Blue for 10 minutes at 37 degrees Celsius. Then, the absorbance was read on a plate reader (BioTeK Synergy H1 microplate reader) and the viability data was collected. The plate reader was set to a temperature of 37 degrees Celsius with a 1-minute slow, orbital shake, as per the Presto Blue assay's manufacturer's instructions. The experiment was repeated at least three

times with both HeLa and HDF cells until there was enough data to significantly confirm our results.

During the second iteration of viability testing, pictures of both cell types at 0 hours (before treatment) and 24 hours after treatment were taken through the microscope using a GalaxyS8 smartphone camera.

Migration Scratch Assay

After 24 hours of incubation time after treatment, the cells in the 48-well plates were observed under a microscope to ensure 90-100% confluency. A vertical scratch through the cells were made down the center of each well of both plates using a micropipette tip. A picture was taken of each well's scratch immediately afterwards. The plates were returned to incubation under standard conditions and subsequent pictures were taken of each well's scratch at 24 hours, 48 hours, and 72 hours. The Wound Healing Size Tool plugin for ImageJ, developed by the University of the Andes in Bogotá, Colombia, [4] to analyze how much regrowth occurred over each period.

Statistical Analysis: Viability Assay

A standard deviation and confidence intervals were calculated using all the collected data to determine if there was a statistically significant difference between the effect of DSE on the viability of HeLa cells versus HDF cells. First, the average of the three data points for each concentration was taken. The averages were used to find the variance at each concentration using the formula show in Figure 4 below. Standard deviation was calculated for each concentration and is the square root of the variance. [5] Using the above calculations, the 95% confidence intervals were found (formula shown

in Figure 5). [6] All steps were repeated for both rounds of viability assays. R was used to calculate P-values using an ANOVA test for both rounds of viability assay.

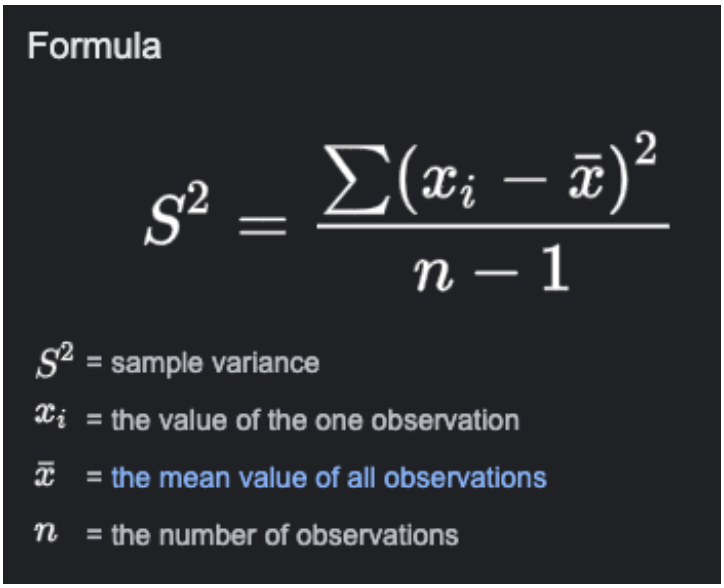


Figure 4: *Formula for variance.*

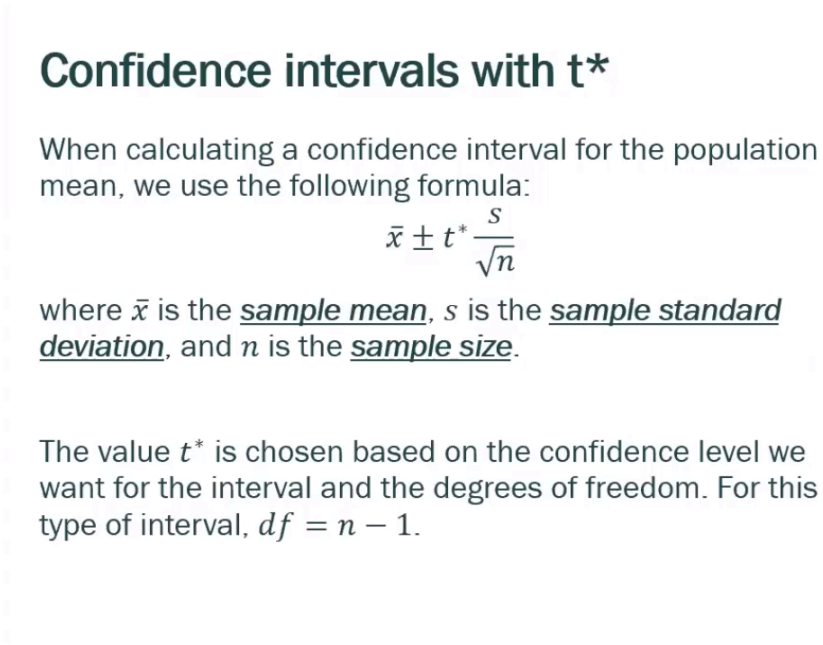


Figure 5. *The formula for calculating confidence intervals. The confidence level chosen was 0.05 as that is the desired level of statistical significance.*

Statistical Analysis: Migration Assay

T-values were calculated for each concentration of DSE at 24-, 48-, and 72-hours. First, the averages were taken of the three data sets at each time interval. Then the variance value of each value was found using the formula shown in figure 4 above. The variance of the HeLa versus the HDF cells were compared at each concentration and time interval by finding the pooled variance (formula shown in Figure 6 below). [7] Next, the averages of the HDF cells were subtracted from the averages of the HeLa cells to see the differences in the means for each value. Using the calculations above, a two sample T-test was run of each value. The T-test formula used is shown before in Figure 7. [8] A T-value significance threshold was found- using a standard T-table [9], degrees of freedom (=sample size-1), and a desired significance value of 0.05- 2.92.

$$s_p = \sqrt{\frac{(n_1 - 1){s_1}^2 + (n_2 - 1){s_2}^2}{n_1 + n_2 - 2}}$$

Figure 6. *Formula for pooled variance. Sp=pooled variance, n1=number of observations in sample 1, s1^2= variance for sample 1, n2=number of observations in sample 2, and s2^2=variance for sample 2.*

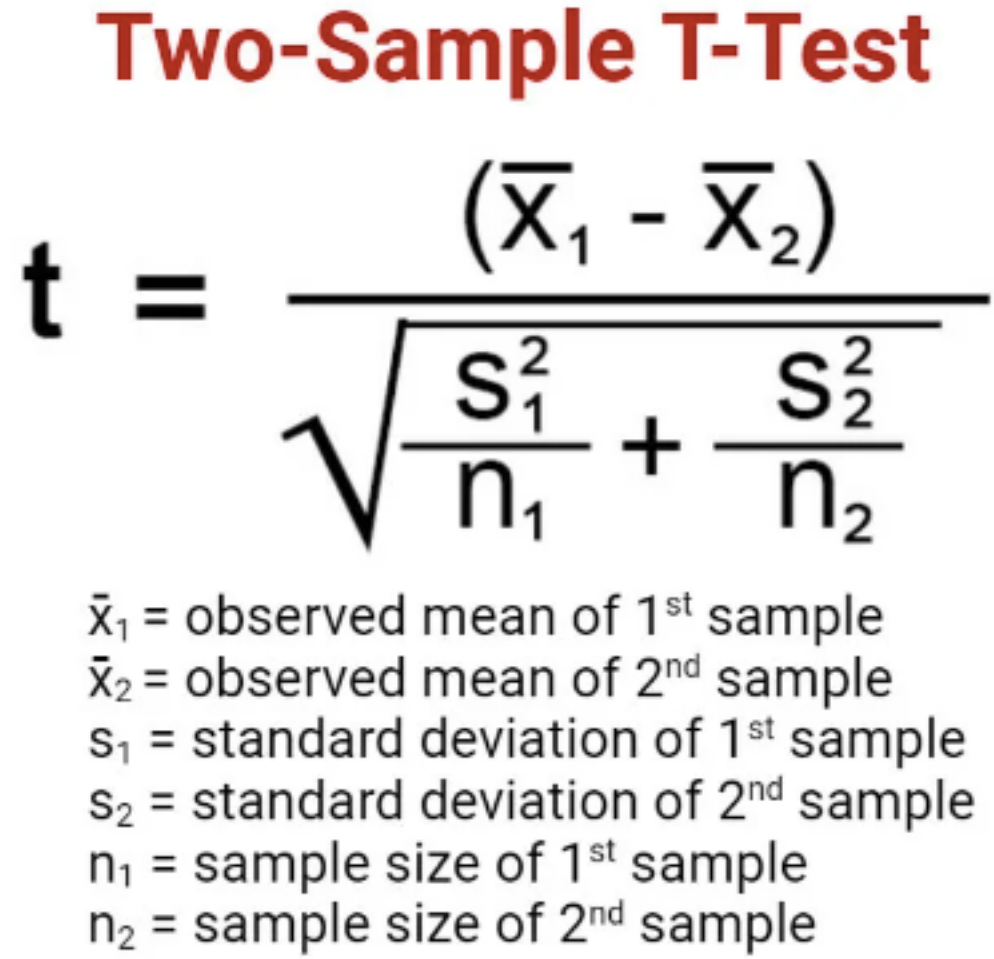


Figure 7. *Formula for two sample T-test.*

Transcriptomics Data Analysis

Transcriptomics is the study of transcriptomes and their functions. Transcriptomes are all the messenger RNA, or mRNA, in a cell, tissue, or organism. [10] mRNA is a molecule that functions as a link between genes- DNA sequences that code for specific proteins- and protein-creating ribosomes in a cell. Transcriptomic data sets are meant to show the changes in gene expression through the types and number of transcriptomes that are present. [11] In this case, this type of analysis was used to determine how gene

expression differed when cells, both cancerous and non-cancerous, were treated with DSE.

A set of transcriptomics data was provided by Dr. Christina Hendrickson of the Biology Department at Oklahoma City University. This data set showed the transcriptomes present in treated and untreated HeLa and HDF cells and how they differed from one another. An analysis of this data set was conducted using the online data processing tool, Galaxy, as well as Microsoft Excel, to determine specific pathways- known series of genes/proteins that interact with one other to cause a change in an organism- affected when these cells are treated with DSE. This type of analysis surveyed over 60,000 genes in the human genome and showed how the DSE affects the extract-treated HeLa and HDF cells. Research into the most affected genes showed that the Wnt pathway was heavily impacted by the DSE treatment and was chosen for further study. Two key genes involved in the Wnt pathway- SFRP1 and Axin2- were evaluated individually to determine DSE's effect on said genes and on their involvement in cancer-causing pathways. Alongside those two genes, a third- BMF- was also chosen for further analysis due to its effect on apoptosis and drastic change in levels of gene expression during treatment. These three genes were further verified through western blot testing.

Using genes that are known to be involved in cancer-causing and/or cancer-preventing pathways, we can get a better look at what this treatment is doing to cancerous and non-cancerous cell lines on a genomic scale. Observing what genes are changed after treatment is administered can provide insight into why behavior changes are seen in other assays

Western Blot

Western blots were conducted to confirm the effects found in the transcriptomics data to specific pathway(s). HDF and HeLa cells were grown in separate 6-well plates until they reached 100% confluency. Proteins were harvested from the cultures and total protein concentration was measured using a spectrophotometer (NanoDrop). To do this, the media was removed from wells and ice-cold PBS wash was added into each well for 5 minutes and then removed. This wash step was repeated a second time before 80uL of a mammalian protein extraction reagent (MPER)+ protease/phosphatase inhibitor mix was added to each well. The inclusion of these inhibitors is to prevent protein dephosphorylation and hydrolysis (break down), functions of phosphatases and proteases respectively, during sample preparation and storage time. [12] The plates were then left on ice for 20 minutes.

After the 20-minute ice bath, each well was scraped, and its entire contents were placed in a 1.5mL tube. All six tubes were then centrifuged for 10 minutes at 12,000 RPM at 4 degrees Celsius. The supernatant of the 1.5mL tubes were transferred into separate 0.6mL tubes. 2uL of the supernatant was loaded onto the NanoDrop immediately and the protein concentration was recorded. The MPER + protease/phosphatase inhibitor mix served as a blank. Proteases and phosphatases are proteins, and their presence in the samples would be canceled out by including them in the blank. Measurements were conducted at room temperature to obtain the optimum accuracy and precision measurements.

NanoDrop protein concentration measurements were used to determine the amount of sample needed to load 35 micrograms of total protein per well (the protein

load recommended by the antibody vendor and literature). Next, SDS-PAGE electrophoresis was set up. 10X Reducing, 4X SB, water, and the samples were mixed into small tubes and spun briefly to combine. Then the samples were heated to 70 degrees Celsius for 10 minutes to denature the proteins and release their bond with any DNA or other debris. This helps to ensure the proteins will be easier to load into the gel. [13]. While the samples were heating, the Invitrogen Bolt 4%-12% Bris-Tris gel running apparatus was assembled using 12 lanes and each lane was flushed with running buffer to remove any debris. Then water was added to each well to help the samples sink to the bottom of the well. After the samples were heated, the whole contents of the tubes were pipetted into each lane. Next, protein markers were loaded into separate lanes, and only one or two lanes were used per gel. The gel apparatus was run at 100-150V for 50-90 minutes until the dye bands approached the bottom. After the electrophoresis was run, the apparatus was dissembled, and a spatula was used to pry apart the plastic casing to obtain the gel. The gel was cut at the top of each column and the entire bottom piece; these parts were discarded. We used Bolt transfer buffer (Invitrogen; Thermo Scientific) in a tray and gently placed the gel into the buffer to equilibrate before transfer.

Protein transfer from gel to protein were conducted using standard Western Blot procedure [14]. Blotting paper, nitrocellulose membranes (Bio-Rad), and polyacrylamide gel were equilibrated submerged in transfer buffer for 10 minutes, then prepared for assembly onto the transfer apparatus. On the lower surface of the Bio-Rad Trans-Blot SD semi-dry transfer cell, the materials were stacked in this order, from bottom (platinum anode) to top (stainless steel cathode): blotting paper, membrane, gel, blotting paper, with a lid placed on top. The membrane needed to be placed under the gel so that when the

negatively charged proteins migrated downward through the gel towards the positively charged anode, they would attach to the membrane below. [15] The transfer was run at 10 volts for 28 minutes. Once the run was complete, the gel was peeled back to ensure the ladder bands had moved onto the membrane. A blocking solution was prepared by mixing 2 parts SuperBlock solution (Thermo Fisher) with 8 parts TBST (**T**ris-**B**uffered **S**aline solution with **T**ween-20).

The membranes were cut vertically into subunits containing the ladder and 2 lanes of the protein samples. The cut membranes were placed into wells and were immediately covered with 10 mL of blocking solution and was rocked for 30 minutes. After 30 minutes, the block solution was poured off. The experimental primary antibody, control primary antibody (Pa) was added to the wells. The membranes were incubated in the presence of the primary antibodies overnight at 4 degrees Celsius on a rocking platform.

The wells were then washed five times with TBST, removing the solution between each wash, to remove any residual unbound antibody from the target protein. The secondary antibody was pipetted into the wells. The plate was then covered in foil and rocked for another 55 minutes, followed by another 5 washings with TBST for 5 minutes each. The membrane was removed and suspended above the blotting paper; capillary action allowed the excess liquid to be removed. Then the membrane was covered with plastic wrap and placed on the Bio-Rad ChemiDoc MP Imaging System face up. The image was collected using the automatic optimized protocol found in the Imaging System's commands.

These methods were repeated for each gene. The primary antibodies used for each gene were as follows: Rabbit monoclonal anti-human SFRP1 (AbClonal catalog #

A9656), rabbit polyclonal anti human BMF (AbClonal catalog # A5796), Mouse monoclonal anti-human GAPDH (1:500; Developmental Studies Hybridoma Bank). Mouse monoclonal anti-human Axin-2 (Conductin), (Santa Cruz catalog # sc-25302). The specific secondary antibodies used were as follows: Goat anti-mouse Alexa 488, 1:5000; goat anti-rabbit Alexa 647 (1:5000).

The polyacrylamide gels run in a 10 well apparatus were set up in the following order, from left to right: 1) ladder 2) HeLa vehicle control (DMSO 2%) 3) HeLa vehicle control 4) DSE treated HeLa 5) DSE treated HeLa 6) ladder 7) HDF vehicle control 8) HDF vehicle control 9) DSE treated HDF 10) DSE treated HDF. The polyacrylamide gels run in a 17- well apparatus were set up in two different ways. The polyacrylamide gels that contained Axin2 and BMF were set up in the following order, from left to right: 1) ladder 2-4) HeLa vehicle control 5-7) DSE treated HeLa 8 and 9) no cells, reagent blank 10) ladder 11-13) HDF vehicle control 14-16) DSE treated HDF 17) no cells, reagent blank. The polyacrylamide gels that contained GAPDH and SFRP1 were set up in the following order, from left to right: 1) ladder 2-4) HeLa vehicle control 5-7) DSE treated HeLa 8) no cells, reagent blank 9) ladder 10-12) HDF vehicle control 13-15) DSE treated HDF 16 and 17) no cells, reagent blank. PageRuler Plus 250 was used as the ladder for all polyacrylamide gels. After all polyacrylamide gels and western blots were conducted, the clearest results were reported in the *western blot results* section below.

Results

Presto Blue HS Cell Viability Assay

After the viability assay was run three times for each cell type, the viability percentage for each concentration of DSE was collected and recorded in Tables 3-6 and

Figures 8 and 9 below. Figures 10 and 11 below show pictures of the cells at 0 hours (before treatment) and at 24 hours after treatment before the viability assay was performed for the second round of viability assay. The control cells that were treated with a 0 mg/ml DSE treatment were set to 100% viability and all other viability percentages were compared to that standard.

Table 3. Recorded viability percentages of HeLa cells treated with DSE: First Round

01/22/22		01/29/22		02/16/22	
DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)
0	100	0	100	0	100
0.2	134	0.1	137	0.1	128
0.5	86	0.2	104	0.2	120
1	1	0.3	98	0.3	92
1.5	0	0.4	58	0.4	75
2	1	0.5	53	0.5	68
n/a	n/a	0.6	3	0.6	63
n/a	n/a	0.8	0	0.8	4

Table 4. Recorded viability percentage of HDF cells treated with DSE: First Round

02/10/22		02/16/22		02/22/22	
DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)
0	100	0	100	0	100
0.1	148	0.1	135	0.1	123
0.2	127	0.2	105	0.2	120
0.3	107	0.3	87	0.3	97
0.4	96	0.4	79	0.4	72
0.5	129	0.5	99	0.5	98
0.6	1	0.6	60	0.6	34
0.8	1	0.8	10	0.8	0

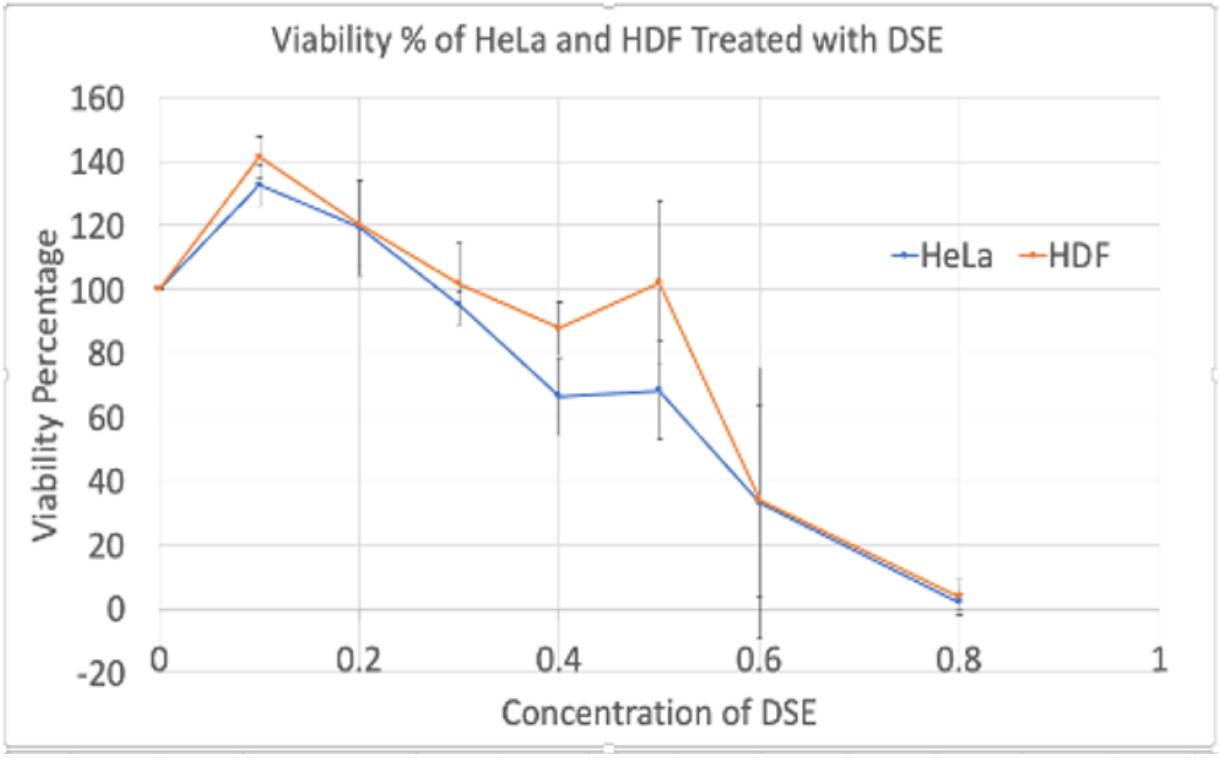


Figure 8. *First round of viability assay graph. DSE treatment reduced viability of HeLa cells to a greater extent than HDF cells. Cells were plated in 96 well plates and treated with the indicated concentrations of DSE. Presto Blue viability assay demonstrated reduced viability with increasing doses in both HDF and HeLa cells, however, HeLa showed greater loss of viability in the mid-range doses tested. Data points are the average of three trials per cell type. Concentration of DSE is in mg/ml.*

Table 5. Recorded viability percentages of HeLa cells treated with DSE: Second Round					
01/07/24 Plate 1		01/07/24 Plate 2		01/07/24 Plate 3	
DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)
0	100	0	100	0	100
0.1	104	0.1	79	0.1	80
0.2	118	0.2	72	0.2	77
0.4	117	0.4	73	0.4	66
0.6	61	0.6	43	0.6	47
0.8	28	0.8	12	0.8	30
1	0	1	1	1	0

Table 6. Recorded viability percentages of HDF cells treated with DSE: Second Round

01/07/24 Plate 1		01/07/24 Plate 2		01/07/24 Plate 3	
DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)	DSE Concentration (mg/ml)	Viability Percentage (%)
0	100	0	100	0	100
0.1	74	0.1	79	0.1	94
0.2	68	0.2	75	0.2	92
0.4	62	0.4	51	0.4	46
0.6	21	0.6	24	0.6	23
0.8	2	0.8	0	0.8	1
1	0	1	0	1	0

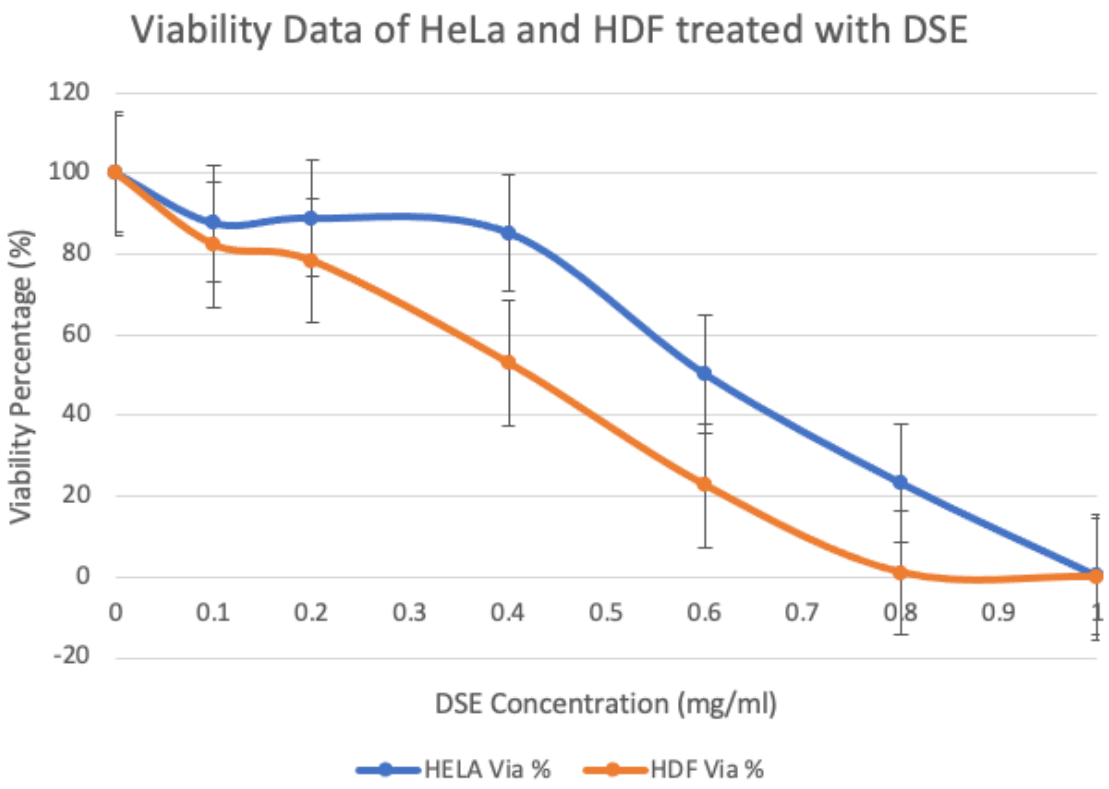


Figure 9. *Second round of viability assay graph. DSE treatment reduced viability of HDF cells to a greater extent than HeLa cells. Cells were plated in 96 well plates and treated with the indicated concentrations of DSE. Presto Blue viability assay demonstrated reduced viability with increasing doses in both HDF and HeLa cells. Data points are the average of three trials per cell type. DSE concentration in mg/ml.*

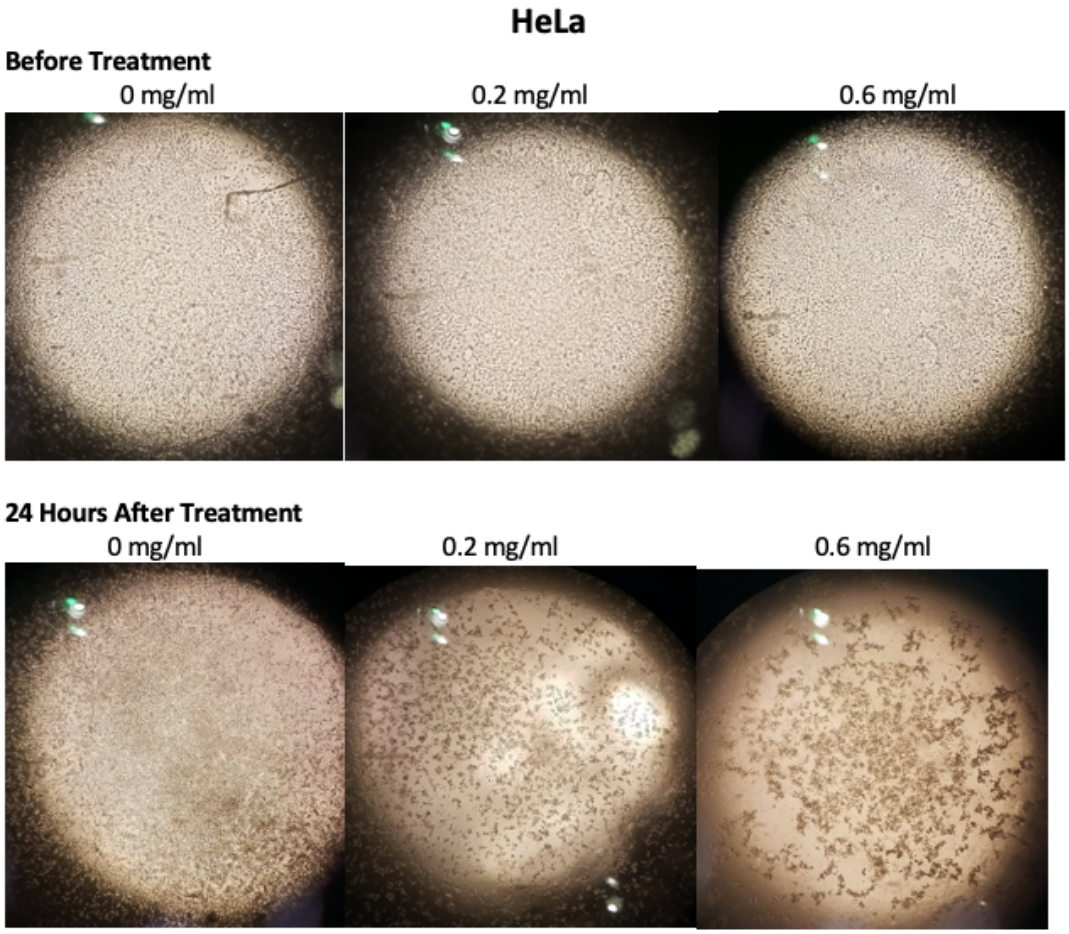


Figure 10. *Photos of HeLa cells before and 24 hours after DSE treatment during the second round of viability assay.*

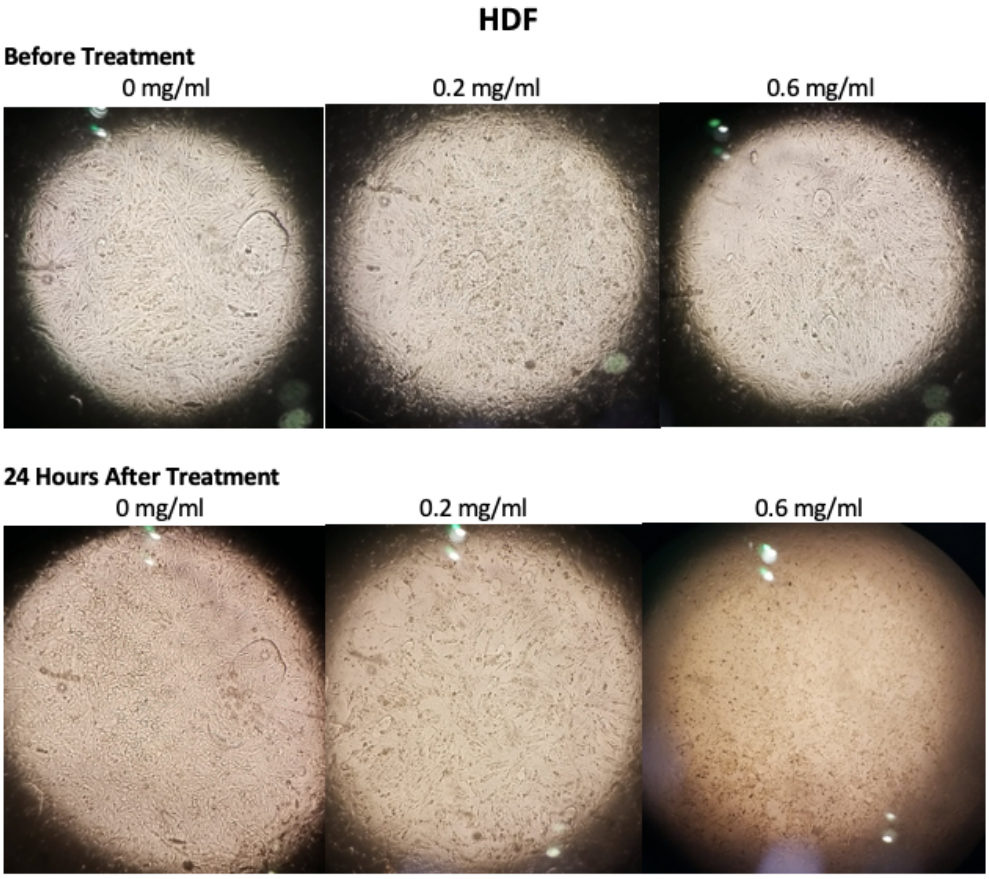


Figure 11. *Photos of HDF cells before and 24 hours after DSE treatment during the second round of viability assay.*

Migration Assay

After the migration assay was run, the percentage of wound healing for each concentration of DSE was collected using ImageJ for 24, 48, and 72 hours. Figures 11 and 12 below depict the average migration percentage for each treatment concentration for HeLa and HDF cells separately. Figure 13 below compares the average migration percentage for each treatment concentration of HeLa and HDF cells at 72 hours. The

control cells that were treated with a 0 mg/ml DSE treatment were set to 100% wound openings and all other wound closure percentages were compared to that standard.

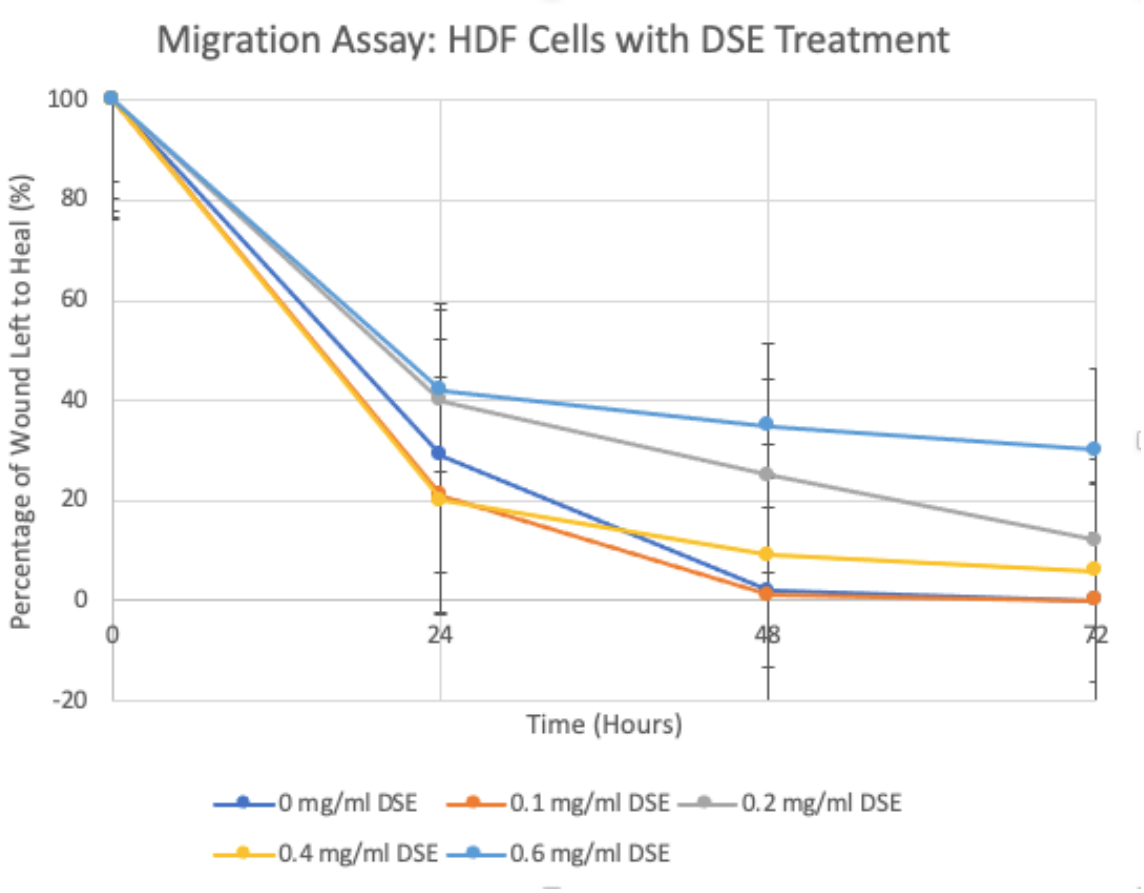


Figure 12. *Graph depicting the average wound closure percentage for each DSE treatment concentration on the HDF cells after 24, 48, and 72 hours. Data points are the average of three trials.*

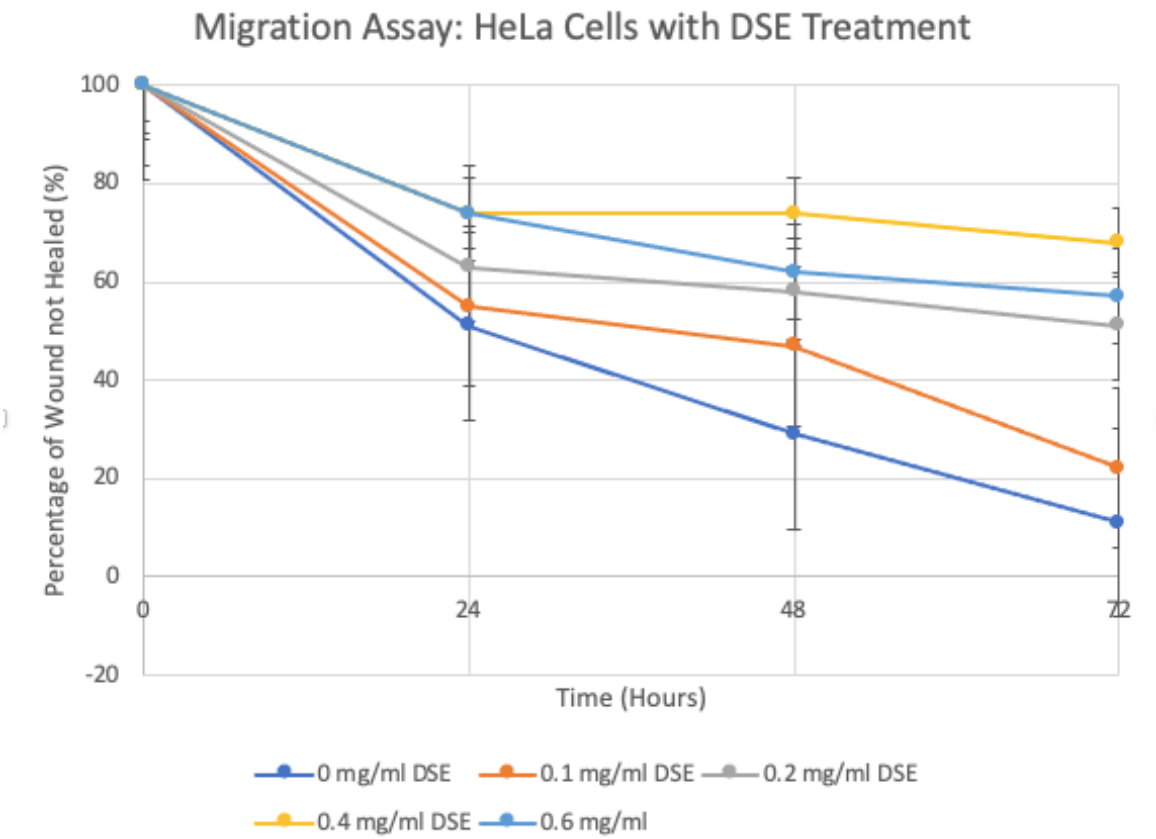


Figure 13. *Graph depicting the average wound closure percentage for each DSE treatment concentration on the HeLa cells after 24, 48, and 72 hours. Data points are the average of three trials.*

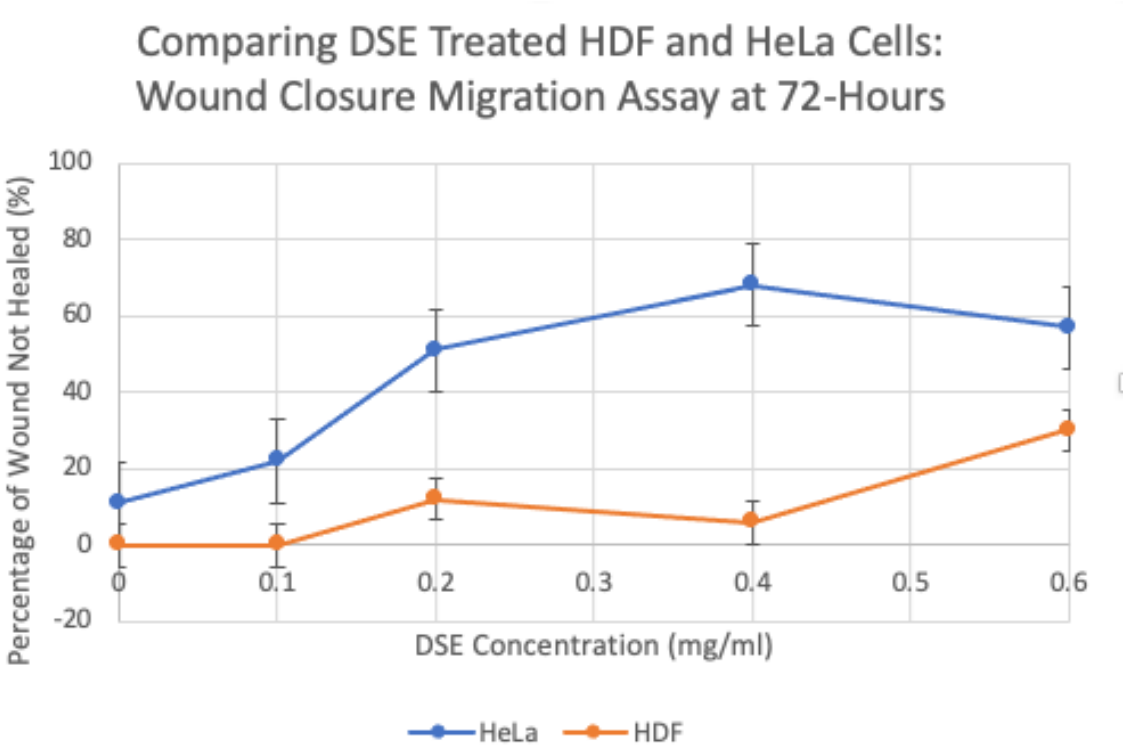


Figure 14. *Graph comparing the average wound closure percentage for each DSE treatment concentration on the HeLa and HDF cells after 72 hours. Data points are the average of three trials per cell type.*

Statistical Analysis: Viability Assay

When an ANOVA test was conducted, three p-values were found: one describing the effect the DSE concentration alone has on cell viability, another describing the effect cell type has on viability, and the third describing the effect the interaction between those two factors has on cell viability. The results from the first round of viability testing can be seen in Figure 13 and the results from the second round of viability testing can be seen in Figure 14. The calculated 95% confidence intervals for each treatment concentration for both cell types and both rounds of viability assay are described below is Tables 7-10.

```
##                     Df Sum Sq Mean Sq F value   Pr(>F)
## Concentration        1  52654   52654  83.711 2.98e-11 ***
## Cell                 1    552     552   0.877    0.355
## Concentration:Cell   1      5       5   0.008    0.927
## Residuals           39  24531     629
```

Figure 15. *P-values calculated for the first round of viability testing results from the ANOVA test. P-value for the effect the DSE concentration has on viability=2.98x10^-11, P-value for the effect the cell type has on viability=0.355, P-value for the effect the interaction between the two factors has on cell viability= 0.927. Symbols to the right of the p-values indicate the significance level as follows: 0-0.001=***, 0.001-0.01=**, 0.01-0.05=*, 0.05-0.1=., 0.1-1=no marking.*

```
##                     Df Sum Sq Mean Sq F value Pr(>F)
## Concentration        1  53674   53674 297.292 <2e-16 ***
## Cell                 1   2086    2086  11.554 0.0016 **
## Concentration:Cell   1     81      81   0.446 0.5082
## Residuals           38   6861     181
```

Figure 16. *P-values calculated for the second round of viability testing results from the ANOVA test. P-value for the effect the DSE concentration has on viability=2.00x10^-16, P-value for the effect the cell type has on viability=0.0016, P-value for the effect the interaction between the two factors has on cell viability= 0.5082. Symbols to the right of the p-values indicate the significance level as follows: 0-0.001=***, 0.001-0.01=**, 0.01-0.05=*, 0.05-0.1=., 0.1-1=no marking.*

Table 7: Calculated Confidence Intervals for Viability Assay Data: HeLa Cells: First Round

DSE Concentration (mg/ml)	Confidence Interval: Low End (%)	Confidence Interval: High End (%)
0	100	100
0.1	113.14	151.86
0.2	82.04	156.63
0.3	85.87	104.13
0.4	29.92	103.08
0.5	27.62	109.71
0.6	-96.09	162.09
0.8	-6.61	10.61

Table 8: Calculated Confidence Intervals for Viability Assay Data: HDF Cells: First Round		
DSE Concentration (mg/ml)	Confidence Interval: Low End (%)	Confidence Interval: High End (%)
0	100	100
0.1	104.27	166.40
0.2	89.41	145.27
0.3	72.16	121.84
0.4	51.67	113.00
0,5	64.90	152.43
0.6	-41.79	105.13
0.8	-10.02	17.35

Table 9: Calculated Confidence Intervals for Viability Assay Data: HeLa Cells: Second Round		
DSE Concentration (mg/ml)	Confidence Interval: Low End (%)	Confidence Interval: High End (%)
0	100	100
0.1	52.50	122.90
0.2	26.30	151.70
0,4	16.65	154.02
0.6	26.85	73.81
0.8	-1.17	47.84
1	-1.10	1.77

Table 10: Calculated Confidence Intervals for Viability Assay Data: HDF Cells: Second Round		
DSE Concentration (mg/ml)	Confidence Interval: Low End (%)	Confidence Interval: High End (%)
0	100	100
0.1	56.48	108.19
0.2	47.68	109.00
0.4	32.66	73.34
0.6	18.87	26.46
0.8	-1.48	3.48
1	0	0

Statistical Analysis: Migration Assay

The T-values and difference in means that were found for each concentration during each time interval comparing both cell types to one another are described in Table 11 below.

Table 11. Calculated T-values for Migration Assay Comparing HeLa and HDF Cells

DSE Concentration (mg/ml)	Difference in Mean: 24-hours (%)	T-Value: 24-hours	Difference in Mean: 48-hours (%)	T-Value: 48-hours	Difference in Mean: 72-hours (%)	T-Value: 72-hours
0	42	5.504	47	8.087	31	4.764
0.1	7	0.439	6	1.046	2	1.000
0.2	23	1.741	33	1.971	39	3.312
0.4	54	4.201	65	4.313	62	4.472
0.6	32	2.702	27	2.867	27	2.097

Transcriptomics Data Analysis

As shown in tables 12 and 13, Axin2 was found to be downregulated about 5-fold in treated HeLa cells and upregulated about 1-fold in treated HDF cells. As shown in the tables below, SFRP1 was downregulated about 3.5-fold in treated HeLa cells and downregulated approximately 1.5-fold in the treated HDF cells.

Finally, BMF was chosen because of the large effect it was shown to have on the treated HeLa cells compared to the treated HDF cells. As shown in tables 12 and 13, BMF was downregulated approximately 127-fold in the treated cancer cells and downregulated only about 9-fold in the treated non-cancerous cells.

Looking at the p-values of the changes seen in all the genes, the significance of the change in the controls versus in the treated cells can be determined. Axin2 is shown to have a p-value of 0.0023 in the HeLa cells and a p-value of 0.5056 in the HDF cells when treated with DSE, indicating that the effect DSE treatment has on HeLa cells is statistically significant and the effect on the HDF cells is not. BMF is shown to have a p-value of 8.74E-06 in HeLa cells and a p-value of 0.00015 in HDF cells when treated with DSE. This indicates that the effect DSE has on BMF in both HeLa and HDF cells is statistically significant. Tables 12 and 13 below show that DSE's effect on SFRP1 is not significant in either HeLa nor in HDF cells, as the p-values are 0.1558 and 0.0911 respectively.

Table 12. Untreated vs DSE treated HeLa cells gene analysis					
Gene Full Name	Gene Common Name	Control Mean	Treatment Mean	Fold Change	P-value
ENSG00000104081	BMF	17.8	0.14	-127.14	8.74E-06
ENSG00000168646	AXIN2	5.02	0.98	-5.12	0.00230233
ENSG00000104332	SFRP1	0.11	0.03	-3.67	0.15580987

Table 13. Untreated vs DSE treated HDF cells gene analysis					
Gene Full Name	Gene Common Name	Control Mean	Treatment Mean	Fold Change	P-value
ENSG00000104081	BMF	7.97	0.89	-8.96	0.0001541
ENSG00000168646	AXIN2	2.42	2.77	1.14	0.50556118
ENSG00000104332	SFRP1	11.75	18.05	1.54	0.09108537

Western Blot

Both cell types were treated with DSE at 0.2 mg/ml and approximately 50% of the HeLa cells survived the treatment, allowing for a successful western blot to be run. Glyceraldehyde-3-phosphate dehydrogenase, or GAPDH, was used as the loading control for these western blots. For the BMF and GAPDH runs, 25 micrograms were loaded, and the 12-well bolt gel was utilized. For the SFRP1 and Axin2 runs, 35 micrograms were loaded, and the 10-well bolt gel was utilized. The difference in loading volumes comes from the differing protein concentrations in each antibody. Using the protein concentration provided by the NanoDrop machine and the desired volume of protein desired, the amount of each antibody was calculated using the following equation: [desired protein mass] / [protein concentration from NanoDrop]. The results of the western blots are shown below in Figures 15-17.

As shown in Figures 16 and 17 in the western blots of Axin2 and BMF, there was a clear downregulation of the genes in the HeLa treatment versus the HeLa control columns for these genes. This is indicated by the faded line weight in the treated cells' column as compared to the thicker, darker line weight in the control cells' column. As shown in Figure 17, in the western blot of SFRP1, there was not a clear difference between the gene presence in HeLa or HDF treated and control cells. This is indicated by the consistent line weight in each column of the western blot.

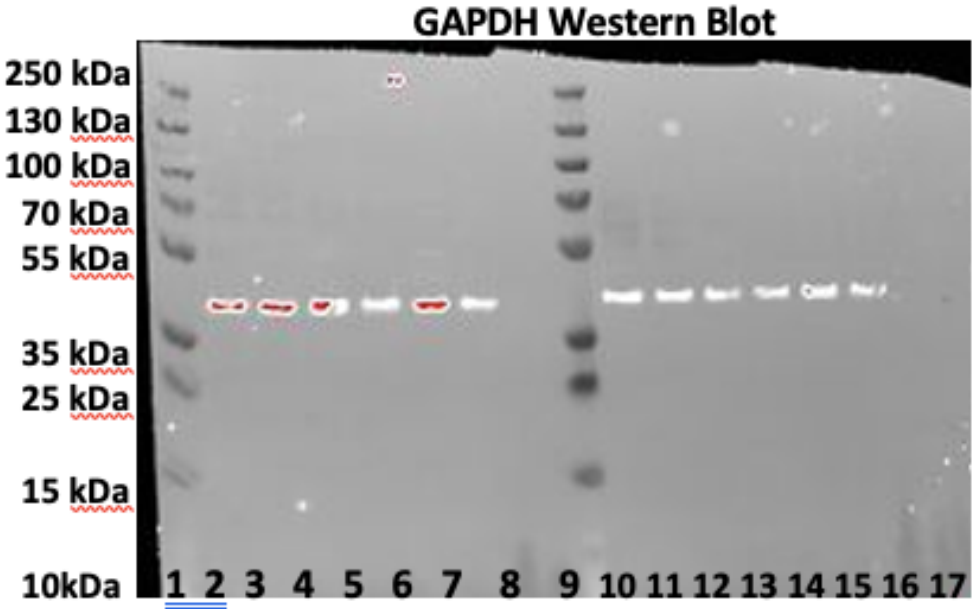


Figure 17. *Western Blot of GAPDH. This was used as a control for this experiment. All lanes are similar in thickness and opacity, indicating consistent protein levels.*

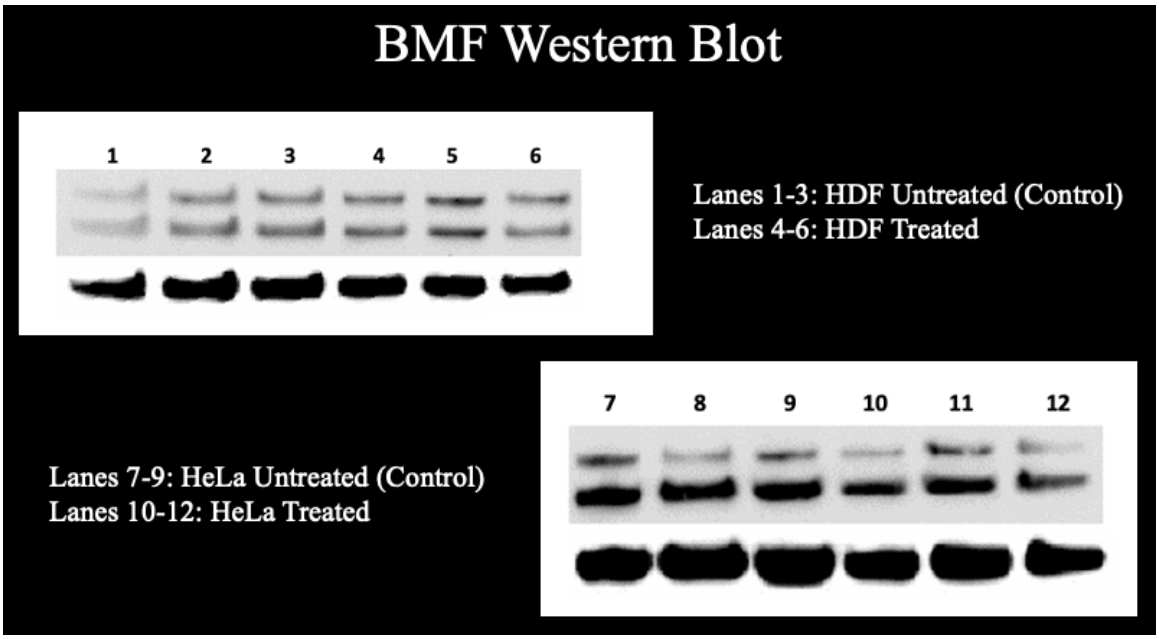


Figure 18. *Western Blot of BMF. The top two rows in each column are the BMF results and the bottom row is the GAPDH control results. The consistent line weight and opacity in the GAPDH line shows that the protein was loaded evenly. The BMF lines are slightly more faded in HeLa treated (lanes 10-12) than in HeLa untreated (lanes7-9) indicating a slight downregulation of BMF in the HeLa cells. There is not much of a visible difference between the BMF line weights of the HDF lanes (lanes1-6).*

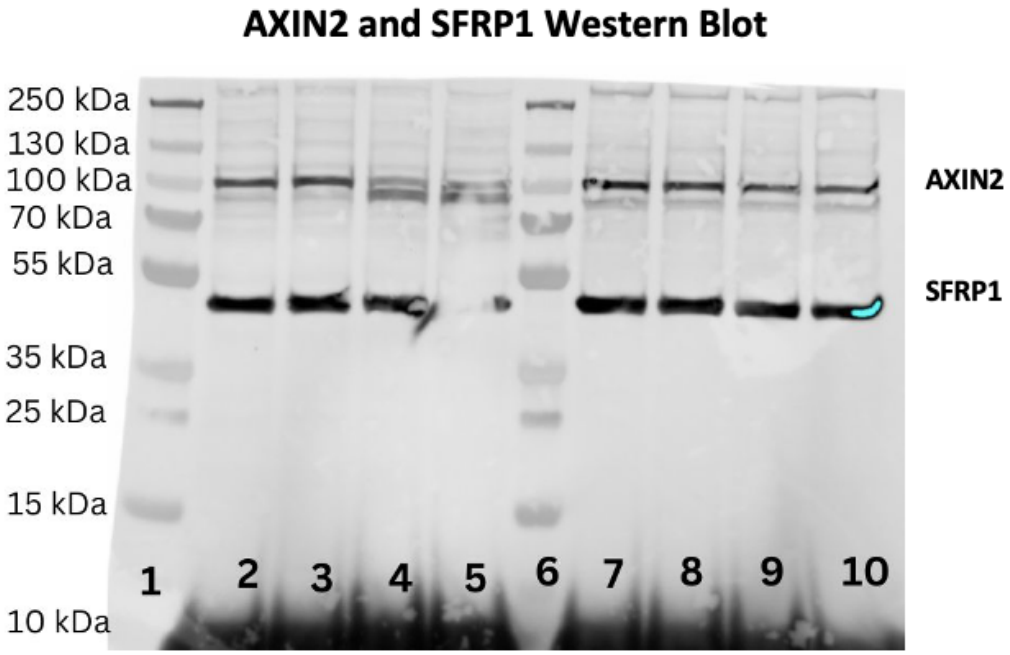


Figure 19. *Western blot of SFRP1 and Axin2. The lanes assignments are the following: 1) ladder 2 and 3) control HeLa cells 4 and 5) treated HeLa cells 6) ladder 7 and 8) control HDF 9 and 10) treated HDF cells. There is a visible difference between the control HeLa cells and the treated HeLa cells and not as much of a difference shown between the HDF cells in the Axin2 run. There is not much of a visible difference between the treated and untreated cells of either kind for the SFRP1 run. One of the treated HeLa lanes (Lane 5) is smeared and cannot be considered. This is likely due to a transfer error, which is often caused by an air bubble between the gel and the membrane.*

Discussion

Presto Blue HS Cell Viability Assay

Cellular respiration is a process done inside a cell to create ATP, an energy source for cells. ATP can be consumed by cells to power functions such as ion transport, muscle contraction, nerve impulse propagation, substrate phosphorylation, and chemical synthesis. [16] When this is done in the presence of oxygen, it is called aerobic respiration. The initial step of aerobic cellular respiration is glycolysis. Glycolysis is an

oxidation-reduction reaction and involves the breakdown of glucose into pyruvate through series of intermediates, which creates energy. This reaction involves NAD+, which is a carrier molecule. NAD+ binds to electrons on hydrogen atoms inside of glucose and carries them to the electron transport chain, changing from NAD+ to NADH when it does this. The electrons are transported to protein complexes that help to create an electrical gradient by pumping electrons across the intermembrane space. When protons travel across the cellular membrane, to neutralize the gradient, they pass through ATP-synthase, powering it to phosphorylate ADP to create ATP, cellular energy. [17] Once the electrons move through this system they attach to free oxygen (O2) molecules, yielding an energy output of -52.5 kcal/mol per pair of electrons. [18] This gaining of electrons is called reduction and the cell is in a reducing environment. This is a sign of a viable cell as it indicates that cellular respiration is occurring.

Presto Blue is a resazurin-based solution that uses the reducing environments of living cells to measure the proliferation of cells. [19] When Presto Blue is added to viable cells, it permeates the membrane of the cell and responds to the reduction occurring inside the cell. When Presto Blue interacts with the electrons carried by NADH, it changes from resazurin, a blue molecule that does not fluoresce, to resorufin, a red and fluorescent molecule. [20] This fluorescence can be detected by absorbance plate readers. The amount of fluorescence will correlate to the number of viable cells that are present.

There is a phenomenon that happens with fibroblasts if they get too confluent called fibroblast contact inhibition. When fibroblasts, including the HDF used here, meet one another, they tend to try to pull apart and stop proliferating. [21] When the HDF cells were treated with the DSE they were approximately 80-90% confluent in each well. This

had the potential to affect the results as the fibroblasts may have been too crowded, slowing proliferation and cellular growth before treatment was administered.

Concentrations of 0, 0.2, 0.5, 1, 1.5, and 2 mg/ml of DSE were chosen for the first run-through of the experiment. However, when it was found that 100% of the cells were dying at 1 mg/ml concentration, all following experiments were run with DSE concentrations less than 1 mg/ml. At first glance, the results of the first round of the Presto Blue viability assay seemed to indicate that there was a range from 0.4-0.6 mg/ml where the cancerous HeLa cells were dying off and the non-cancerous HDF cells were surviving. This was the ideal range that was needed to be found for a potential treatment to move into having clinical applications. However, once the statistical analysis was performed, it became apparent that the gap was not large enough to have significance. The calculated p-value for this round was 0.927 and a p-value above 0.05 is considered not statistically significant.

Low concentrations of DSE treatment seemed to cause an increase in cell viability compared to the controls in cancerous and non-cancerous cells. The mechanism of this result is unknown, but one probable cause has to do with the antioxidant properties of dandelions. When cells are grown in an artificial lab environment, they can be put under a lot of additional stressors compared to when they grow naturally in an organism. It has been shown that small doses of antioxidants can help relieve some of these stressors on artificially grown cells. [22] Dandelions have been shown to have naturally occurring antioxidant properties [23], which could account for low doses of dandelion extracts could help cells grow and overcome stressors more than non-treated control cells. This result differs from micro-doses of other chemotherapy drugs. Multiple studies have tested

micro-doses of popular chemotherapy drugs, including nivolumab, doxorubicin (DOX), etoposide (ETOP), and busulfan, and found that micro-doses have similar anti-cancer properties as their higher dose counterparts [24, 25, 26].

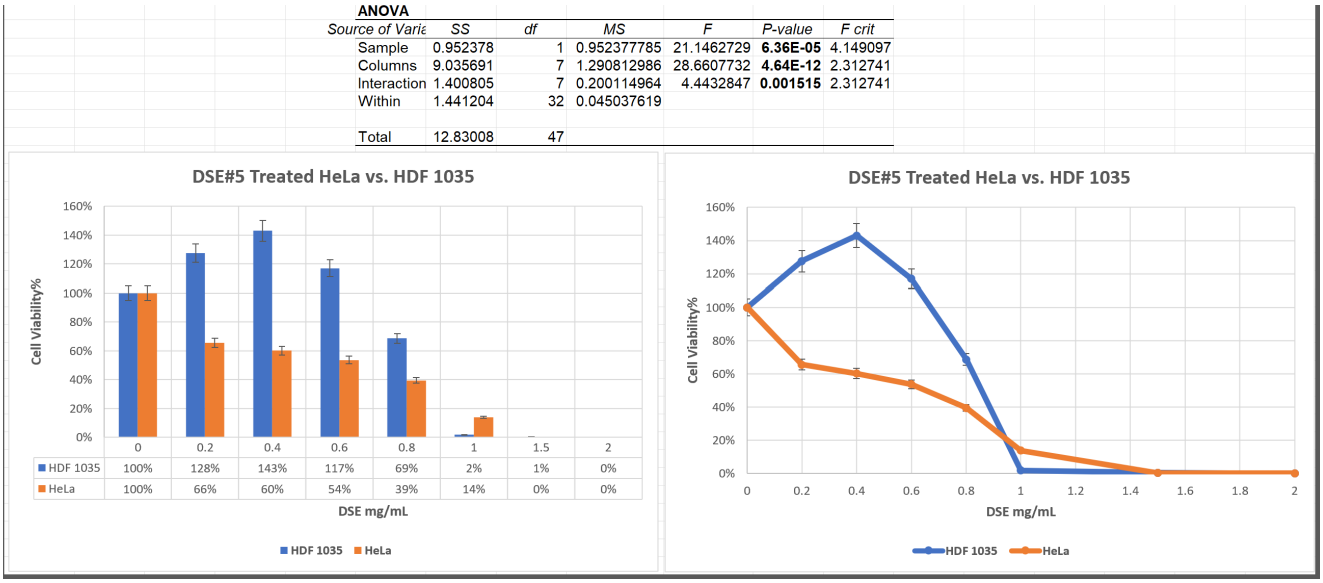
The results of the second round of the Presto Blue viability assay does not support the hypothesis that this method of DSE treatment decreases the viability of cancerous cells more drastically than the viability of non-cancerous cells. However, like the first round of testing, the calculated p-value for the test was 0.5082, which is also above the significance threshold and therefore not statistically significant either.

As shown in figures 10 and 11, when the cells were observed under the microscope, it appears that the treated HeLa cells were unhealthier than the treated HDF cells, especially at higher DSE concentrations. However, when the viability percentages were measured, the opposite result was shown. Resazurin-reduction viability assays- including Presto Blue- work by measuring signs of healthy cells, like metabolism. As described above, Presto Blue works by reading the amount of resazurin being turned into resorufin by a cell in active metabolism. [27] It is probable that the DSE treatment is more aggressively affecting the metabolism of HDF than HeLa, causing the cell viability to be reportedly lower in HDF cells, despite the HeLa cells looking less healthy under the microscope. Another probable cause is that the treatment is killing off more of the HeLa cells, but the surviving cells are still metabolizing at a higher rate than the surviving HDF cells. [28]

As shown in Figure 20 below, similar experiments with the same cell type can have different results. This can be due to a few factors. Phenotype drifting is a phenomenon that occurs in cells that are cultured for a long period of time. Over time cell

lines can lose tissue-specific functions and act in a way that is very different than those cells *in vivo*. [29] The longer cell lines are grown, the more they can change and the more likely that they are no longer representative of the broader cell population. A 2018 study conducted by the Broad Institute of Harvard and MIT, found that drug interactions between cells from the same cell line can be drastically different; out of 321 anti-cancer compounds, at least 75% that strongly impacted some strains were completely inactive in others. [30]

Varying cell lines can also play a role. Cell lines originate from primary cells that are taken from a specific organism. [31] For example, the specific HDF cell lines used in here, 00703 and 01035, came from two separate individual humans, each with their own demographic history. These variations can lead to differences in the phenotypes expressed by cell lines of the same cell type. Despite their ease and inexpensiveness, the use of immortal cell lines versus primary cells has its draw backs. Cell lines are suspectable to changes and have been found to differ from their primary counterparts over time. [29]



ANOVA

Source of Varia	SS	df	MS	F	P-value	F crit
Sample	0.952378	1	0.952377785	21.1462729	**6.36E-05**	4.149097
Columns	9.035691	7	1.290812986	28.6607732	**4.64E-12**	2.312741
Interaction	1.400805	7	0.200114964	4.4432847	**0.001515**	2.312741
Within	1.441204	32	0.045037619			
Total	12.83008	47				

Figure 20. *Viability assay data provided by Dr. Hendrickson's lab at Oklahoma City University. There was a significant difference between the effect of DSE on the viability of HDF cells (cell line:)1035) and HeLa cells. p-value=0.001515. These results differ from the second iteration of viability testing reported in figure 9 above, which also used HDF 01035.*

Statistical Analysis: Viability Assay

Confidence intervals are used provide a range of mean value around the sample mean that the true population is likely to fall in. The wider the spread of a confidence value, the less accurate the experimental mean is likely to be when compared to the true population mean as it covers too wide of a range of values to be precise. It has been determined to be a better measure for more real-world conclusions about clinical relevance. [32] If the confidence intervals of the HeLa and HDF treatments overlap, they are considered less statistically significant at that concentration due to the possibility of

having a matching true population mean, which would indicate a lack of difference in the reaction to the treatment.

As shown in Tables 7-10 above, the confidence intervals of the first round of viability assays heavily overlap with one another. They also show very large gaps for most of the concentrations. This suggests that this treatment would likely struggle to be differentially effective in a clinical setting between cancerous and non-cancerous cells in a meaningful way. This is backed up by the high p-value the calculations on this treatment provided.

The second round of viability testing shows more real-world promise than the first iteration. The confidence intervals are much smaller ranges and they do not overlap between the cell types as drastically as in the first tests. This implies that these results are likely to reflect a more realistic estimation of the effect this treatment would have if used on a larger scale with more complex models.

Migration Assay

Late-stage cancers, or metastatic cancer, is defined by the movement of cancerous cells, usually through blood and/or lymph systems, from their original tumor location to other organs of the body. [33] The further and more widespread the cancer has moved around the body before treatment, the worse the prognosis is for the patient. For example, as of 2023, the 5-year survival rate of someone diagnosed with localized early-stage cervical cancer is 91%, while someone whose cancer has spread to nearby tissues is 60%, and when the cancer has spread to distant parts of the body is only 19%. [34] Slowing or stopping the movement of cancerous cells can help to minimize the rate of metastasis.

Statistical Analysis: Migration Assay

The specific test used was a one tail two sample T-test. For these tests, the null hypothesis is that the two population-means are equal, and the alternative hypothesis is that HDF's mean cell mobility is higher than HeLa's mean cell mobility under treatment. Once the hypotheses were established, T-values were calculated. If the T-value was greater than the significance threshold- in this case 2.92- then the null hypothesis was rejected and the alternative hypothesis was supported by the data. [35] The T-values show that a concentration is statistically significant and the difference in the mean shows the magnitude of the difference.

At 24-hours, the 0.4 mg/ml DSE treatment had a T-value of 4.201 and the control had a t-value of 5.504, indicating both concentrations had a significant result. The difference in mean was larger with the 0.4 concentration (54%) versus the control (42%), showing that 0.4 had a larger effect on migration than the control. At 48-hours, there were similar results, as the control and 0.4 concentration showed significance with t-values of 8.087 and 4.313 respectively and the 0.4 treatment showed a larger effect with a higher mean difference. At 72-hours, the control, 0.3 and 0.4 all showed significance with t-values greater than the threshold. 0.4 continued to have the largest impact with a mean difference of 62%, while 0.3 had a difference of 39% and the control had a difference of 31%.

These results show that 0.4 mg/ml DSE concentration treatment was able to significantly slow the migration of HeLa cells more than HDF cells at 24-, 48-, and 72-hours. 0.3 mg/ml DSE concentration treatment also demonstrated the ability to

significantly slow cancerous cell migration more than non-cancerous cell migration at 72-hours.

Transcriptomic Data Analysis

When the data set was received, it was organized from the most changed genes to those least affected. When the data was further analyzed, it became clear that genes involved with the Wnt pathway were heavily affected by the DSE treatment. The genes of the Wnt pathway were investigated further and two key genes, SFRP1 and Axin2, were picked out for further study using western blots. A third gene, BMF, was chosen for further analysis as it was found to be one of the most heavily affected genes in DSE treated HeLa cells, while largely unaffected in the HDF cells.

Axin2, also known as Conductin, plays a role in the phosphorylation and degradation of β-catenin, an important gene involved in the Wnt pathway. [36] Axin2 is a part of a group of genes called the destruction complex. When the Wnt pathway is not activated the destruction complex attaches to and phosphorylates and ubiquitinates beta-catenin, inhibiting it. Ubiquitination is the process of ubiquitin bonding to the surface of a protein and, in this case, marking beta-catenin for proteosome degradation. [37] The DSE treatment was shown to downregulate Axin2 in HeLa cells more so than in HDF cells. When Axin2 is downregulated, the destruction complex is downregulated; beta-catenin is no longer inhibited and activates the cancer-causing Wnt pathway. These results are not always consistent in practice because the deregulation of beta-catenin has also been found to cause several malignances despite it inhibiting the Wnt pathway. [38]

SFRP1, or Secreted Frizzled Related Protein 1, is associated with the protein Frizzled, which plays an important role in the Wnt pathway. Frizzled is a protein located

in the surface of plasma membranes of Wnt-responsive cells. When Wnt binds to Frizzled it becomes activated, which leads to the phosphorylation of another membrane-bound protein, LRP. The phosphorylated LRP draws the destruction complex away from beta-catenin allowing it to activate the Wnt pathway. [39] SFRP1 is made up of two domains, one of which, the cysteine-rich domain (CRD), is homologous with the Wnt-binding site of Frizzled receptors, working as a competitive inhibitor to Frizzled activation and, in turn, Wnt pathway activation. [40] The downregulation of SFRP1 causes the increased activation of Frizzled and, therefore, the increased activation of the Wnt pathway.

Bcl2 modifying factor (BMF), as the name implies, interacts with Bcl2, a known anti-apoptotic gene that inhibits the intrinsic pathway of apoptosis. Apoptosis is a type of programmed cell death that can be useful in the destruction of specific cell types, like cancerous cells, without harming surrounding cells and tissues. There are two pathways that are used to initiate apoptosis: extrinsic and intrinsic. To initiate the intrinsic pathway, Bid will detect cellular damage and activate Bax. The activation of Bax causes the release of Cytochrome C which will bind with Procaspase 9 and Apaf, activating Procaspase 9 (now known as Caspase 9). Caspase 9 activates the Caspase Cascade, which activates Caspase 3 and targets proteins that tell the cell to undergo apoptosis. [41] Bcl2 is anti-apoptotic because it binds to Bax and restricts the release of Cytochrome C. [42] BMF is pro-apoptotic when activated in two ways: directly activating Bax and suppressing the anti-apoptotic proteins at the mitochondria (including Bcl2). [43] The DSE treatment caused a down-regulation in BMF in HeLa cells more aggressively than in HDF cells, implicating less apoptotic activity in the cancerous cells.

During the Transcriptomics data analysis phase, SFRP1's effect on HeLa cells was determined to not have a low enough p-value to be statistically significant. This was likely due to the control and treatment mean values being too low. Despite the apparent statistical insignificance of this treatment on this gene, SFRP1 was still chosen for further analysis because of its role in the Wnt pathway and the potential for a visual result when put through a western blot. As seen in the below *western blot results* section, SFRP1's western blot did not produce a readable down- or upregulation result and therefore that hypothesis was unsupported.

Western Blots

Western blots show the concentrations proteins present in a sample. This can be used to observe if a certain protein is being inhibited or enhanced by a specific manipulation of the cells. This helps to confirm the results that were found by analyzing the transcriptomics data and gives them more validity.

The purpose of a ladder in a western blot is to mark the molecular weight of the samples. Ladders contain around 10 different proteins that have known molecular weights. When they are run through the electrophoresis step, the proteins cause bands at certain intervals. This makes a sort of "ruler" that can be used to estimate the molecular weight of the unknown sample by noting what bands they line up with in the ladder. PageRuler Plus 250 was chosen as the ladder in this experiment. It is a mixture of nine blue-, orange-, and green-stained proteins ranging from 10 to 250 kDa. [44] As all tested proteins and controls used fall into this size range, it was determined to be an adequate ladder.

The HDF 00703 and HeLa cells were originally treated with 0.6 mg/ml of DSE. In this experiment, all the HeLa cells died before the western blot could be run and the results were inconclusive. To resolve this, a treatment of 0.2 mg/ml of DSE was used, resulting in about 50% of the HeLa cells surviving the treatment. All future western blots were performed at this concertation as it allowed there to be enough cells to run the western blot. GAPDH was chosen as a control for all western blots run. GAPDH is a key enzyme in glycolysis and is widely considered to be a universal internal control for western blots [45]. GAPDH is known as a "housekeeping protein" because it occurs in almost every tissue and has a very stable expression. This makes it a good candidate for a loading control to ensure that all the lanes have a consistent amount of protein loaded in each. [46] Protein concentration was calculated using the NanoDrop and then confirmed by the GAPDH loading control. This confirmation is needed because the NanoDrop is susceptible to contamination, calibration discrepancies, and human loading and mixing errors. [47]

According to the transcriptomics data above, the expected results from the western blots were to see a large decrease in the treated HeLa versus the HeLa control and a small decrease in the treated HDF versus the HDF control in the BMF run. The BMF western blot confirmed the results of the transcriptomics data analysis, which indicated that the effect DSE has on both HeLa and HDF cells is statistically significant, meaning that DSE is expected to cause decreased apoptosis in both cancerous and non-cancerous cells.

The transcriptomics data indicated that there would not be a large difference between the HeLa controls and treatments and the HDF controls and treatments. It was

still suspected that there would be a visible difference between the HeLa control lanes and treatment lanes if a western blot was run as discussed in sections above. That hypothesis was not supported as the SFRP1 western blot did not show a visible difference in the HeLa or HDF controls and treatment.

The western blots of BMF and Axin2 show a second, fainter line around some of the indicator lines of the genes. Multiple banding does not have one clear cause and it is very difficult to pin-point exact causes other than through trial and error, making small changes and conducting many western blots until another result is reached. Common problems include poor handling of the samples, degradation of the sample, too high of a concentration used, and poor-quality antibodies. Proper blocking is important to prevent antibodies binding to the membrane nonspecifically. [48]

The gathering of the transcriptomics data of this DSE treatment and the confirmation of said data with the western blots has helped to paint part of a bigger picture of what this is doing to cancerous cell lines versus non-cancerous cell lines at a transcriptomic level. The effects the DSE treatment had on the three genes highlighted demonstrate that DSE treatment is affecting cancer-related gene pathways inside the cell. The manipulation of the Wnt pathway and the induction of apoptosis in cancerous cells can be a powerful tool in the fight against cancer.

Conclusion

In review, the Presto Blue viability assay results show that there was not a significant difference between the effect of this DSE treatment had on cancerous and non-cancerous cells. This does not support the hypothesis that DSE treatment would have a significant effect on the viability of HeLa cells versus HDF cells. This result is not

necessarily a negative. It can be just as valuable to discover what does not work as it is to discover what does work. This can save time and money with future research. These experiments show that future investigation into this topic is still needed. Other members of the research group run by Dr. Melville Vaughan at the University of Central Oklahoma studied the effects this DSE treatment had on other aspects of the cell cycle, like cellular proliferation. Their experiments yielded varying results with some promising steppingstones for further studies. Additional research has also been conducted by Dr. Christina Hendrickson's team at Oklahoma City University. Their experiments yielded a result that showed DSE treatment significantly reduced the viability of HeLa cells compared to HDF cells. Their team also looked at the anti-microbial properties of DSE. It was shown that DSE treatment at 0.2 mg/ml reduced the viability of *Acinetobacter baumannii* by about 70% and 93% at a concentration of 0.8 mg/ml. This continues to prompt the need for additional studies to be conducted so the true medicinal potential of dandelion seed extracts can be established and expanded upon.

The migration assay results show that DSE treatment at certain concentrations as was able to significantly slow the migration of cancerous cells versus non-cancerous cells. Slowing the spread of cancer cells can help to keep patients in lower, more treatable stages of cancer progression for longer. This can buy them more time for proper diagnosis and treatment and can also help to make treatments easier and more survivable. Further testing into the anti-migration properties of dandelion extracts is warranted.

Through the transcriptomics data analysis, it was shown that DSE treatment influences a few key genes, by causing the downregulation of the Axin2 gene in DSE-treated HeLa cells and the aggressive downregulation of BMF. The western blot results

confirmed and solidified the above results for Axin2 and BMF. The western blot results did not confirm the visible downregulation of SFRP1. It is important to acknowledge that the isolation of three genes does not show the full picture of what the DSE treatment is doing inside of the cells. It can only provide insight into what the treatment is doing to those specific genes and can hint at what might be happening to pathways that those genes are involved in. Additional research will be needed on larger scales and with more complex organisms before the true, real-life effect of this treatment can be claimed.

Proving dandelion extracts have clinical anti-cancer effects could take decades. The Wnt pathway was chosen to be more closely investigated in this study, but there are thousands of other genes that could be studied further and there are many different aspects of cellular life and function to be studied as well, including cell proliferation, apoptotic cell ratios, cell migration, cell cycle stage analysis, etc.

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