

Quantitative Analysis of Aloe-Emodin-Induced Cytotoxicity in MCF-7 Breast Cancer Spheroids and Metabolic Activity Assessment via XTT Assay and ImageJ Morphometric Evaluation

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Background

- Breast cancer is the second leading cause of cancer-related deaths worldwide.
- Current treatments such as chemotherapy, radiation, and surgery are effective but highly invasive and damaging to healthy cells.
- Aloe-emodin is one of the lead bioactive compounds found in Aloe Vera. It is derived from the leaves and gel of Aloe Vera. Aloe-emodin has many properties including antioxidant, anticancer, and anti-inflammatory properties. Pure Aloe-emodin has a bright orange color.
- Previous studies showed that Aloe-emodin induced apoptosis, led to cell death, inhibited breast cancer cell proliferation, and overall acted well as an anticancer agent.

Research Objectives

- Investigate the effects of Aloe-emodin on MCF-7 breast cancer spheroids.
- Measure changes in metabolic activity using the XTT assay.
- Analyze changes in spheroid size and morphology using ImageJ.
- Compare treated spheroids to untreated and Dimethyl sulfoxide control groups.
- Determine whether Aloe-emodin produces cytotoxic and morphometric effects that support its potential as a less invasive complementary therapy.

Assumptions

- MCF-7 breast cancer spheroids accurately represent tumor structure, cellular interactions, and metabolic behavior found in human breast cancer.
- Aloe-emodin is successfully and evenly delivered to each spheroid.
- The XTT assay provides a reliable measurement of cellular metabolic activity.
- ImageJ software accurately measures spheroid diameter, volume, and morphological changes.
- All spheroids begin the experiment with similar cell health.
- Observed effects are primarily due to Aloe-emodin.

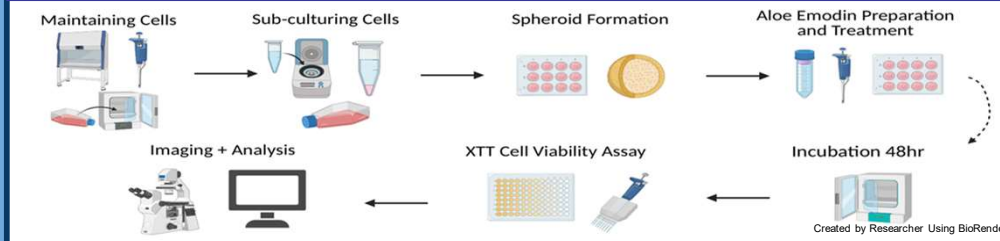
Limitations

- The study uses only one breast cancer cell line, limiting generalization to other cancer subtypes.
- Short-term incubation periods do not reflect long-term or repeated treatment effects.
- In vitro spheroid models do not fully replicate the complexity of tumors in living organisms.
- Variability in spheroid formation may affect consistency between samples.
- Results cannot be directly translated to clinical use without further animal and human studies.

Spheroid Measurement with ImageJ

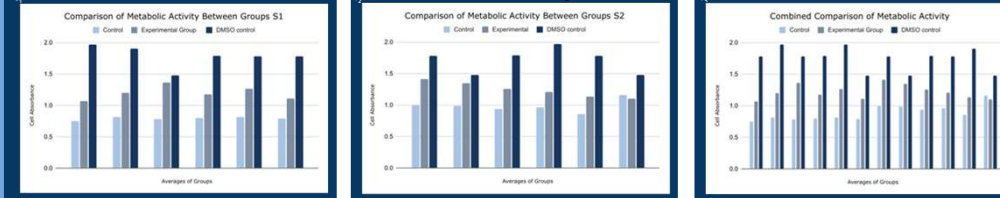
- Open spheroid image in ImageJ.
- Select threshold changing to black and white.
- Outline spheroid using polygon tool.
- Analyze particles and record spheroid size.

Methods

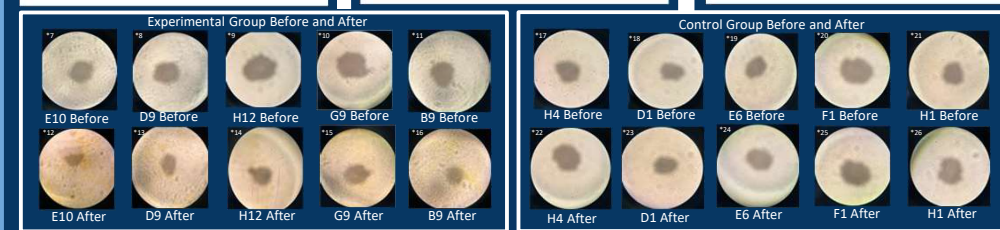
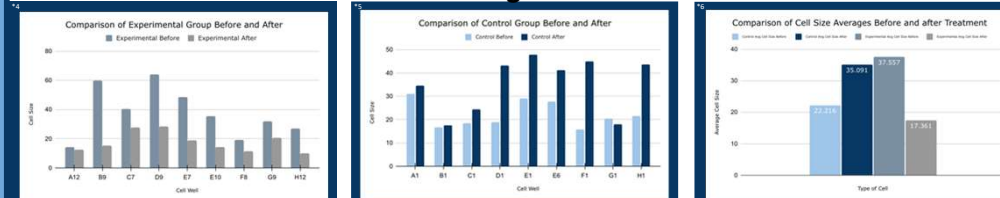


Experiential Outcomes

XTT Assay



ImageJ



Statistical Analysis

❖ Measure of Metabolic Activity Via XTT Assay					❖ ImageJ Analysis of Spheroid Size					❖ Changes in Cell Size									
Spheroid Group One					Before and After Control Cell Sizes					Before and After Experimental Cell Sizes					Control vs. Experimental				
Control	Experimental	DMSO control	Control	Experimental	DMSO control	Control Size Before	Control Size After	Experimental Size Before	Experimental Size After	Control	Experimental	Control	Experimental	Control	Experimental				
0.751	1.069	1.961	0.991	1.409	1.780	A1	31.039	A1	34.543	A1	3.504	A12	-1.908						
0.815	1.203	1.068	0.796	1.261	1.481	B1	16.57	B1	17.665	B9	59.503	B9	14.932	-44.271					
0.779	1.366	1.481	0.936	1.258	1.779	C1	18.510	C1	24.919	C7	40.117	C7	27.456	-12.861					
0.801	1.179	1.779	0.962	1.205	1.967	D1	18.963	D1	43.302	D9	63.954	D9	28.028	-35.920					
0.816	1.296	1.788	0.854	1.133	1.780	E1	29.118	E1	47.807	E7	48.158	E7	18.458	-29.3					
0.787	1.108	1.720	1.107	1.103	1.481	E6	27.772	E6	41.239	E10	35.094	E10	13.914	-51.18					
						F1	19.876	F1	44.688	F8	19.847	F8	11.261	-7.598					
						G1	20.525	G1	17.963	G9	31.724	G9	20.296	-11.428					
						H1	31.566	H1	43.749	H12	26.530	H12	6.743	-18.794					
ANOVA					ANOVA					ANOVA									
Between Groups	12.99620463	df	2	6.83124814	138.473203	0	3.1090911			Between Groups	12.87566667	df	1	-20.19622222					
Within Groups	9.735402209	df	306	0.0474897687						Within Groups	128.5668667	df	9	196.4115987					
Total	20.09199286	df	308							Total	141.4425633	df	10	176.6314874					
ANOVA Test: Single Factor					ANOVA Test: Single Factor					ANOVA Test: Single Factor									
Source of Variation	SS	df	MS	F	P-value	F crit				Source of Variation	SS	df	MS	F	P-value	F crit			
Between Groups	12.99620463	2	6.498102315	138.473203	0	3.1090911				Between Groups	2594.350135	3	864.7833783	6.697860774	0.0012310003	2.901115588			
Within Groups	9.735402209	306	0.0474897687							Within Groups	4131.62785	32	129.1133703						
Total	20.09199286	308								Total	6725.977985	35							
ANOVA Test: Single Factor					ANOVA Test: Single Factor					ANOVA Test: Single Factor									
Source of Variation	SS	df	MS	F	P-value	F crit				Source of Variation	SS	df	MS	F	P-value	F crit			
Between Groups	12.99620463	2	6.498102315	138.473203	0	3.1090911				Between Groups	12.87566667	1	12.87566667	20.19622222	0.0003031726	4.75526297			
Within Groups	9.735402209	306	0.0474897687							Within Groups	128.5668667	9	14.28520741	294.9869664	0.0000063452	2.901115588			
Total	20.09199286	308								Total	141.4425633	10							
ANOVA Test: Single Factor					ANOVA Test: Single Factor					ANOVA Test: Single Factor									
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Total	20.09199286	308								Total	141.4425633	10							

Discussion

- The XTT assay was used to measure cellular metabolic activity through absorbance values.
- Initial analysis showed differences among control, DMSO control, and experimental groups; a one-way ANOVA indicated a statistically significant difference ($p = 0$).
- Post-hoc t-tests confirmed significant differences between the control and experimental groups ($p = 0$).
- Because Aloe-emodin was dissolved in DMSO, the experimental group was also compared to a DMSO control.
- A t-test comparing the DMSO control and experimental group showed a significant difference ($p = 0.000732$).
- These results indicate that Aloe-emodin reduced metabolic activity and suggest that DMSO influenced XTT assay outcomes.
- Spheroid diameters were measured using ImageJ before and after treatment, and size change was calculated for each spheroid.
- Differences in spheroid size change were observed between control and experimental groups.
- A one-way ANOVA showed a significant difference in size change ($p = 0.00123$), which was confirmed by a post-hoc two-sample t-test assuming unequal variances ($p = 0.0000606$).
- Control spheroids generally increased in size, while experimental spheroids decreased, indicating inhibited spheroid growth.
- Together, the reduction in metabolic activity and spheroid size support that Aloe-emodin induced cytotoxic effects in MCF-7 breast cancer cells.
- Overall, the combined XTT and ImageJ results demonstrate that Aloe-emodin significantly affects cell viability and spheroid structure, supporting its potential as a complementary or less invasive cancer treatment.

Implications

- Scientific: Investigates the effects of Aloe-emodin on the growth and viability of breast cancer spheroids, providing insight into its potential anti-cancer mechanisms.
- Medical: May offer a gentler, complementary treatment option that targets cancer cells while minimizing harm to healthy tissue.
- Societal: Plant-based therapies like Aloe-emodin could improve access to cancer treatments in low-resource or underserved areas.
- Economic: Could reduce healthcare costs and encourage further research into affordable, naturally derived anticancer compounds.

Key References

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