SYNERGISTIC ANTICANCER ACTIVITY OF CANNABINOIDS AND TERPENES AGAINST TRIPLE NEGATIVE BREAST CANCER RESISTANCE

Mounika Aare, Islauddin Khan, Aakash Nathani, Breana Boirie, Arvind Bagde, Sukhmandeep Kaur, Mandip Singh*

College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee ,FL 32307, USA

PURPOSE

Cancer is the leading cause of mortality worldwide and 90% of cancer mortality rate is associated with resistance.

Combination therapy, a treatment modality that studies the effect of two or more drugs is the most common strategy used to overcome drug resistance in cancer. Cannabinoids were proven to be potential anticancer agents. Terpenes were shown to exhibit anticancer activity.

The purpose of this study was to evaluate the effect of combinations of various cannabinoids and terpenes against cancer resistance.

HYPOTHESIS

The hypothesis of this study was anticancer activity of minor Phytocannabinoids such as CBC, CBG, CBN, CBDA, CBDV can be further potentiated by combination approach using terpenes

OBJECTIVES

Screen several cannabinoids, terpenes and their combinations against resistant cancer cell lines to get the most potential combination.

1. *In-vitro* and *In-vivo* testing of the obtained combination followed by mechanistic studies.
2. To study the mechanism underlying the synergistic activity of combinations through western blotting and RT-PCR.

METHODS

- *In-vitro* screening of several cannabinoids and terpenes using MTT assay.
- Screening of combinations of cannabinoids and terpenes.
- Cell proliferation assay of screened combinations in 3D spheroids
- Isobolographic analysis to determine synergistic combinations.
- Other techniques such as colony formation, wound healing, cell cycle analysis by flow cytometry on the combinations screened
- *In-vivo* studies on CBC+BC in athymic nude mice
- Studies to evaluate the underlying mechanism such as western blotting and RT-PCR

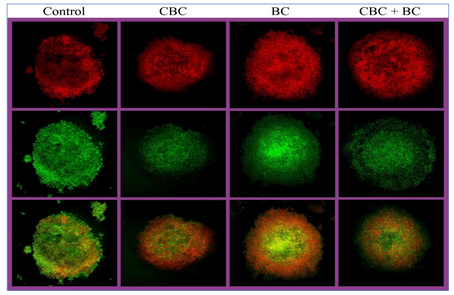
RESULTS

SUMMARY OF CYTOTOXICITY STUDIES

S.NO	CMD	PDX LUNG CANCER CELLS		MDA MB 231 DOX RT CELLS		H1975 OSM RT CELLS	
		2D	3D	2D	3D	2D	3D
1	CBC	7.25± 0.77	20.91±0.28	7.13±0.94	14±0.85	7.41±0.37	23.74±0.74
2	CBG	9± 0.84	28.62±0.38	8.84±0.59	20.42±0.85	9.31±0.42	46.94±0.83
3	CBN	11.9± 0.92	25.29±0.51	8.23±0.23	25±0.93	11.4±0.68	32.56±0.61
4	CBDA	9.3± 0.35	45.83±0.52	9.36±0.22	42.5±0.85	10.58±0.36	29.51±0.37
5	CBDV	8.08±0.95	32.45±0.22	9.36±0.22	35.5±0.62	11.35±0.47	35±0.64
6	BC	32.47± 0.85	90.49±0.40	40.2±0.76	83.5±1.93	37.83±0.62	87.52±0.85
7	CBC+BC	0.74± 0.23	5.37±0.92	0.57±0.02	5.74±0.98	0.73±0.04	5.28±0.26
8	CBG+BC	1.34± 0.05	8.28±0.81	1.04±0.05	8.39±0.53	1.04±0.09	8.47±0.19
9	CBDA+BC	0.8± 0.25	7.24±0.74	1.35±0.07	6.91±0.84	1.73±0.12	7.55±0.35
10	CBDV+BC	1.14± 0.28	6.84±0.59	2.24±0.14	6.78±0.44	2.2±0.17	9.27±0.38

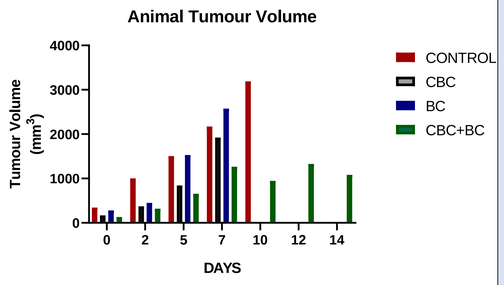
In-vitro cytotoxicity effect against Cannabinoids, Terpenes and Combinations against MDA MB 231 DOX RT, H1975 OSM RT and PDX Lung cancer cells in a 3D spheroid Assay. Results were expressed as Mean ± SD (n=4).

APOPTOSIS ASSAY



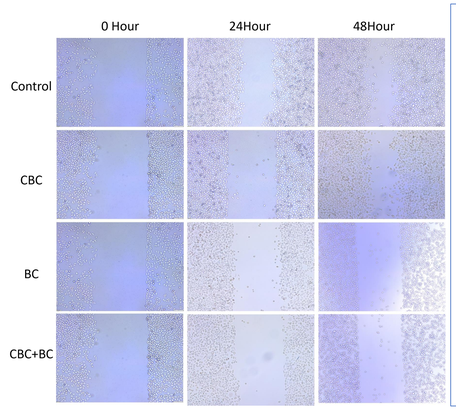
Dual Acridine Orange/Ethidium Bromide fluorescent staining of MDA MB 231 DOX RT Cells. The cells were treated with (a) Control, (b) IC50 concentration of CBC, (c) IC50 concentration of BC and (d) IC50 concentration of CBC+BC. Bar graph representing relative green/red intensity (% of control) P value<0.0001

IN-VIVO STUDIY



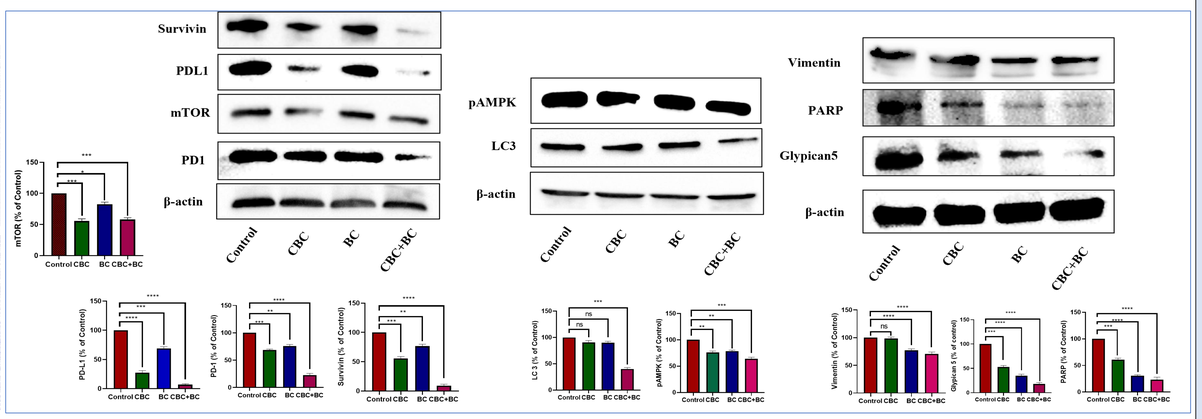
Treatment of MDA MB 231 DOX RT Xenograft models with CBC+BC significantly reduced the tumor volume relative to individual treatments. Tumor volume was measured for every 2 days by using digital Vernier caliper. The results were described as mean ± SD (n = 6)

MIGRATION ASSAY



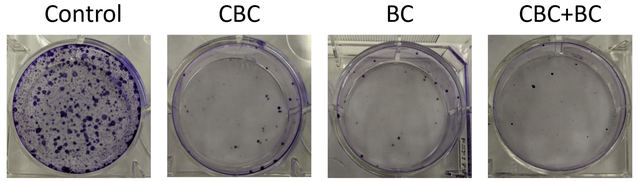
Wound Healing assay. Effect of combination of cannabinoids and terpenes on the cell migration ability of cancer cells was evaluated by wound healing assay.

WESTERN BLOT ANALYSIS OF TUMORS



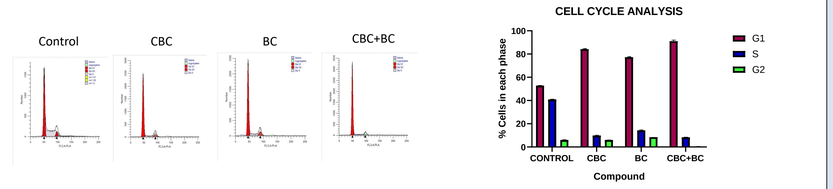
Representative western blots and quantification of respective PD1, PD-L1, Glypican 5, Survivin, pAMPK, Vimentin, Integrin, mTOR, LC 3, PARP in tumor tissue lysate. The values are expressed as Mean ± SD (n = 3). *p< 0.05, **p< 0.01 and ***p< 0.001 vs control.

COLONY FORMATION ASSAY



The Colony Formation of MDA MB 231 DOX RT cells treated with CBC, BC and combination of CBC+BC.

CELL CYCLE ANALYSIS



Cell cycle analysis. Effect of CBC, BC and combination of CBC+BC on cell cycle of MDA MB 231 DOX RT cells. The cells were treated with compounds for 48 hours and cell cycle analysis was done using Flow cytometry. Results were expressed as µM in Mean ± SD (n=4)

CONCLUSIONS

a) From the 2D cytotoxicity studies, it was observed that CBC along with BC was the most potent combination with combination index value of 0.1 for all the concentrations tested. This was further validated by 3D spheroids assay.
b) From all the other *In-vitro* studies such as wound healing, colony formation, apoptosis, cell cycle analysis, it was determined that CBC+BC was the most potent combination.
c) In vivo tumor xenograft studies with resistant MDA-MB231 demonstrated that CBC+BC combination could reduce the tumor volume by more than 4 folds as compared to control.
d) It can be concluded that Cannabinoids and terpenes work synergistically through cell cycle arrest, inhibition of migration and induction of apoptosis against DOX resistant MDA-MB231.

ACKNOWLDGEMENT

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NIH