Blueberry Extract as a Melanoma Therapeutic

Barsha Shrestha, BS; Biplov Sapkota, DVM; Naveen Chintala, PhD; Shilpa Thota, PhD; Rizwana Begum, PhD; Joseph Francis, PhD

LSUHSC School of Medicine, New Orleans, LSU Vet Med Comparative Biomedical Sciences, Baton Rouge

Introduction:

- Although comprising only 5% of skin cancers, melanoma exhibits an aggressive nature, as its tragically responsible for over 75% of skin cancer-related deaths[1].
- The antioxidant, anticancer, and anti-inflammatory nature of blueberries has been extensively investigated, gathering significant attention in research.
- Blueberries possess mechanisms that contribute to cancer prevention by suppressing the production of inflammatory molecules, reducing oxidative stress, and enhancing apoptosis.
- In the realm of cancer therapies, Doxorubicin is widely utilized to treat various cancer forms. Its mechanism of action involves binding to DNA, ultimately inhibiting topoisomerase II and hindering the growth of cancer cells[2] .
- Organoids, which are three-dimensional cell cultures, serve as *in-vitro* models that closely mimic tumors, that allows the monitoring of transformations within the tumor and its environment, advancing our understanding of cancer biology.

Methods:

- A-375 cells, derived from the skin tissue of a 54-year-old female patient with malignant melanoma were maintained and cultured in DMEM medium with fetal bovine serum and Penicillin Streptomycin at 37°C
- A-375 cells were treated with increasing doses of Blueberry Extract and Doxorubicin. At the time points of 24h, 48h, and 72h, 20 µL of MTT dye were added to each well and measured at 570 nm.
- On a 6-well plate, unicellular organoids were formed with the use of Matrigel and A-375 cells. Cells were treated with 5 µM and 1 µM of Doxorubicin, and 2 mg/mL and 1 mg/mL of Blueberry Extract.
- Multicellular organoids were formed with combinations of A-375 cells, HUVEC, and HF-1 cells and then treated with Blueberry Extract 10 mg and Resveratrol 5µM.

Effect of Combination Treatment on A-375 Organoids:

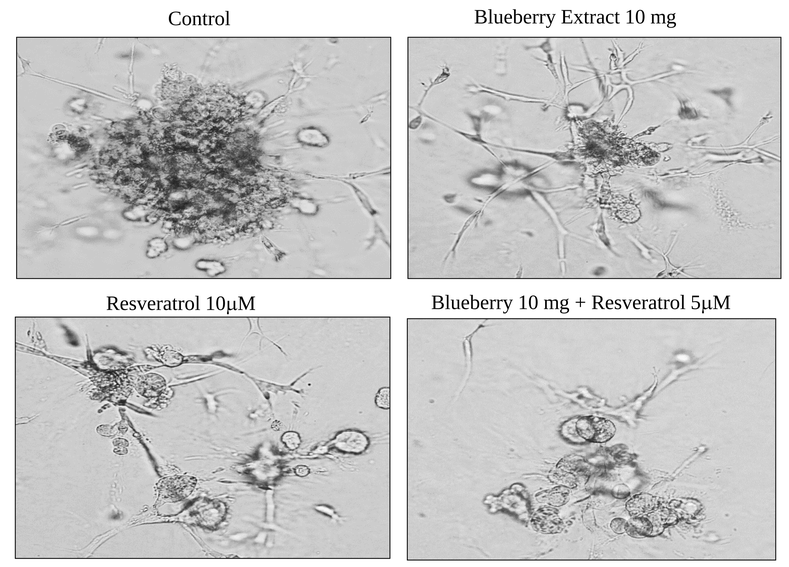
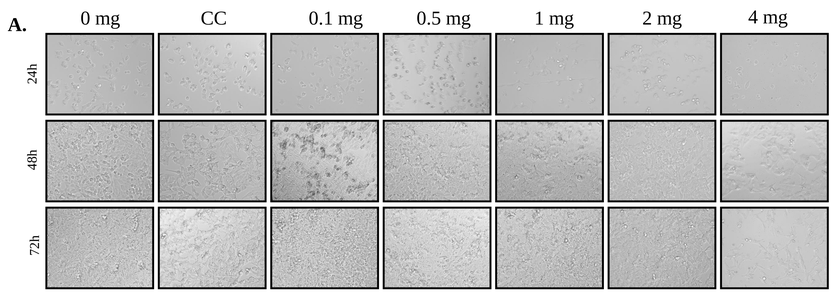


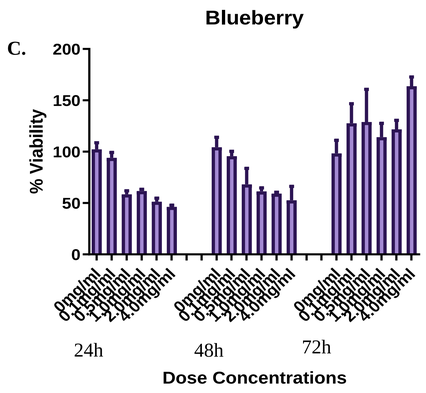
Figure 5: The effect of combination treatment on A-375 organoids: Representative bright field images taken at 20x following the treatment of A-375 organoids with either: Blueberry Extract 10mg/ml, Resveratrol 10µM, and combination of Blueberry Extract and Resveratrol. Following the drug treatment, the organoids tend to disintegrate as compared to the control, suggesting the cytotoxic effect of the Blueberry Extract either alone or in combination with Resveratrol.

Results:

MTS Assay- Cell Viability:

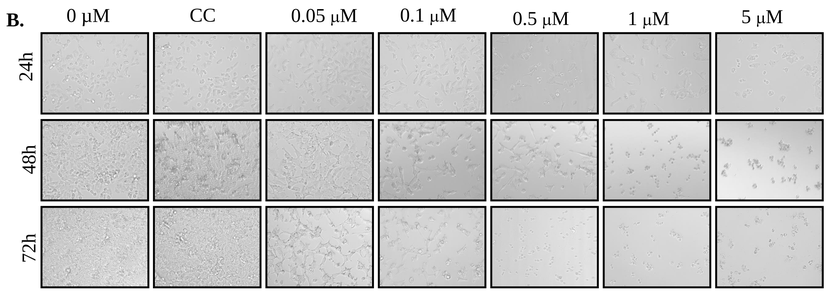
Blueberry:

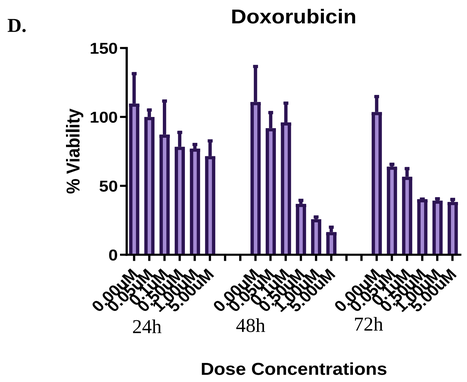




Time	IC50
24h	2.247 mg/mL
48h	3.642 mg/mL
72h	---

Doxorubicin:





Time	IC50
24h	48.63 µM
48h	0.4158 µM
72h	0.2728 µM

Figure 1: Treatment of A-375 with Blueberry Extract and Blueberry. Fig 1A. Bright field images at 20x of A-375 cells following treatment by Blueberry Extract taken at 24h, 48h, and 72h. **Fig 1B**. Bright field images at 20x of A-375 cells following treatment by Doxorubicin at 24h, 48h, and 72h. **Fig 1C.** Cell Viability and IC50 following treatment by Blueberry Extract. **Fig 1D.** Cell viability and IC50 following treatment by Doxorubicin.

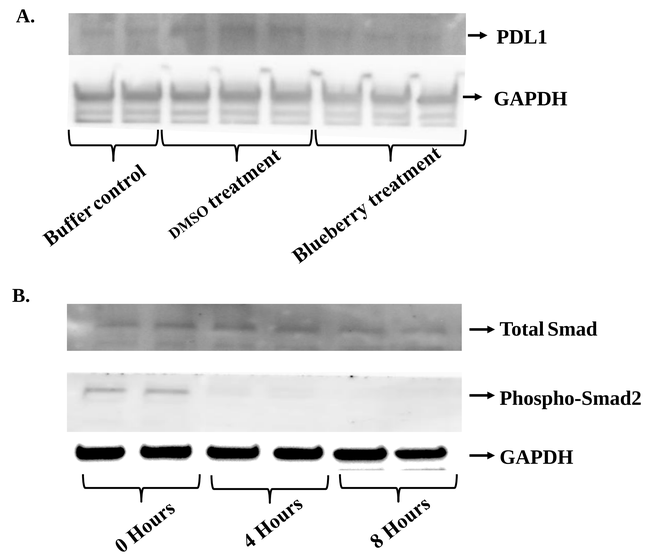


Figure 6: Effect of Blueberry treatment on A-375 cells: Western blot image showing the (**6A**) downregulation of programmed death ligand-1 (PDL-1) following blueberry treatment (**6B**): Following blue berry treatment there is a decrease in the phosphorylated Smad2, while the total Smad did not change. GAPDH was used as the loading control.

3D Cultures:

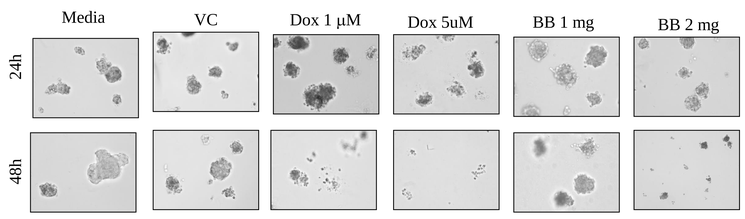


Figure 2: Representative brightfield images of A-375 organoids following drug treatment. Bright field images at 20x of A-375 cells following treatment by Doxorubicin and Blueberry Extract taken at 24h and 48h.

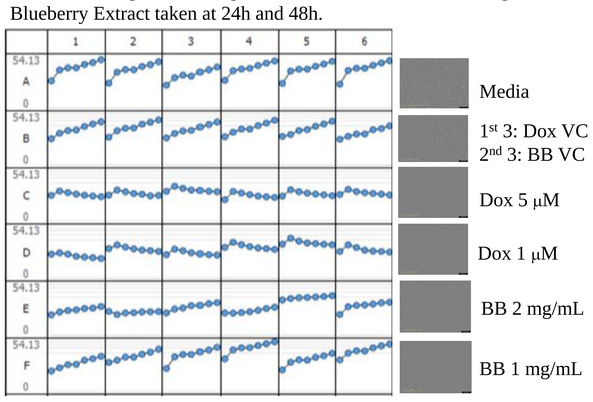


Figure 3: Representative growth pattern for A-375 spheroids following drug treatment. Growth curves of A-375 cells following drug treatment. Media and VC show a linear growth as indicated in the growth curve, unlike the drug treatments where the cells fail to exhibit the linear growth pattern.

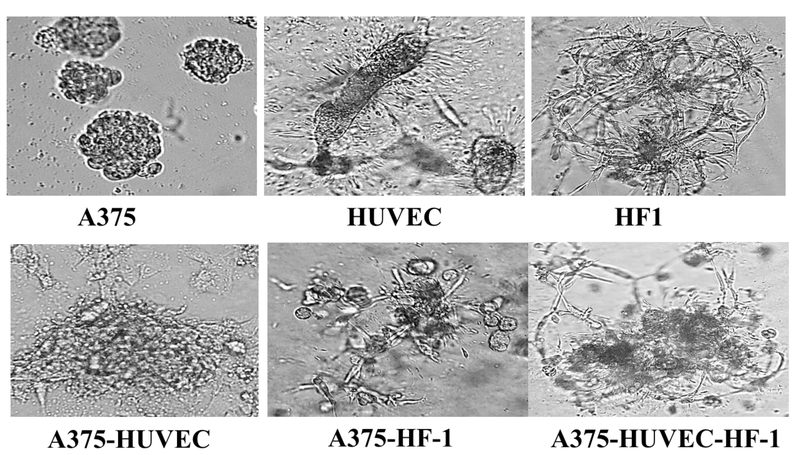


Figure 4: Development of multicellular A-375 organoids. Representative brightfield images taken on day 6, magnification 20x of the multicellular organoids formed by culturing A375 cells, HUVEC (human umbilical vein endothelial cells), and HF-1 (human fibroblast) respectively in the presence of basement membrane extract. These multicellular organoids represent a unique system to interrogate the cellular crosstalk within the tumor microenvironment.

Conclusions:

In this study, we demonstrated the effect of Blueberry Extract to reduce the cell viability of A-375 cells *in vitro*. The observed cytotoxic effect of the extract was confirmed in both the 2D- and 3D cultures. Also, Doxorubicin treatment of A-375 cells demonstrated significant anticancer activity. The relevance of the 3D-tumor microenvironment was established by the development of multicellular organoids including A-375 cancer cells, HF-1 fibroblast, and HUVEC endothelial cells grown as cell suspension in a matrigel. We further demonstrated that these multicellular organoids could be used for drug testing. Interesting, blueberry treatment downregulated the PDL-1 (immune checkpoint inhibitor) and pSmad2 suggesting the role of blue berry in regulating the immunological and anti-inflammatory pathways in A-375 cells. In the future, a combination of Doxorubicin and Blueberry Extract would be tested for a potential therapy against A-375 cells *in vitro*. Furthermore, this combination therapy may be tested in *in vivo* models of mouse melanoma.

References:

(1). Rebecca, V.W., Somasundaram, R. & Herlyn, M. Pre-clinical modeling of cutaneous melanoma. *Nat Commun* **11**, 2858 (2020). https://doi.org/10.1038/s41467-020-15546-9.

(2). Baciu, D.D., Dumitrașcu, A.M., Vasile, V. et al. Generation of a 3D melanoma model and visualization of doxorubicin uptake by fluorescence imaging. In Vitro Cell.Dev.Biol.-Animal 58, 44–53 (2022). https://doi.org/10.1007/s11626-021-00636-9.