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Elderberry (*Sambucus nigra*) Shows Promise as a Naturopathic Treatment Against Melanoma *in vivo* and Several Elderberry Fractions Decrease Melanoma and Neuroblastoma Cell Proliferation *in vitro*

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INTRODUCTION

Melanoma causes an estimated 71-80% of skin cancer deaths due to its aggressive metastatic nature in late stages of the disease. Incidence rates of melanoma continue to grow annually, suggesting the need for preventative anti-melanoma research.

Naturally occurring dark-pigmented berries may have such cancer-inhibiting properties. There is evidence that elderberry extracts can be incorporated into endothelial cells to protect against oxidative stress¹ and stimulate an immune response that may suppress tumor growth².

Our goal is to identify active elderberry fractions capable of modulating proliferation of melanoma both *in vivo* and *in vitro*, and to assess the ubiquity of fraction activity in other cancer cell lines. Proper identification of tumor-suppressing elderberry fractions may lead to diet-based strategies for the prevention of many cancers, including melanoma.



Figure 1. Elderberry (*Sambucus nigra*)



Figure 2. A human melanoma lesion

METHODS

C57BL/6J mice were randomly grouped into a water treated (control) group and an elderberry (10 mg/mL) treated group. Mice were given daily 0.5 mL i.p. treatment injections for 14 days before s.c. injection of 1×10^5 B16-F10 murine melanoma cells to the right flank. Treatments were continued up to day 21 and mice were sacrificed on day 27.

Column Chromatography was used to separate the components of elderberry powder. Fractions were evaporated dry and re-dissolved in 0.5 mL PBS. Neighboring active fractions were pooled.

Human melanoma (MeWo), murine melanoma (B16-F10) and human neuroblastoma (SH-SY5Y) cells were treated with 10 μ L crude and pooled fraction treatments at 24 hours. Cells were tagged with 1 mCi/mL radioactive tritiated thymidine at 48 hours and were harvested and counted at 72 hours.

RESULTS

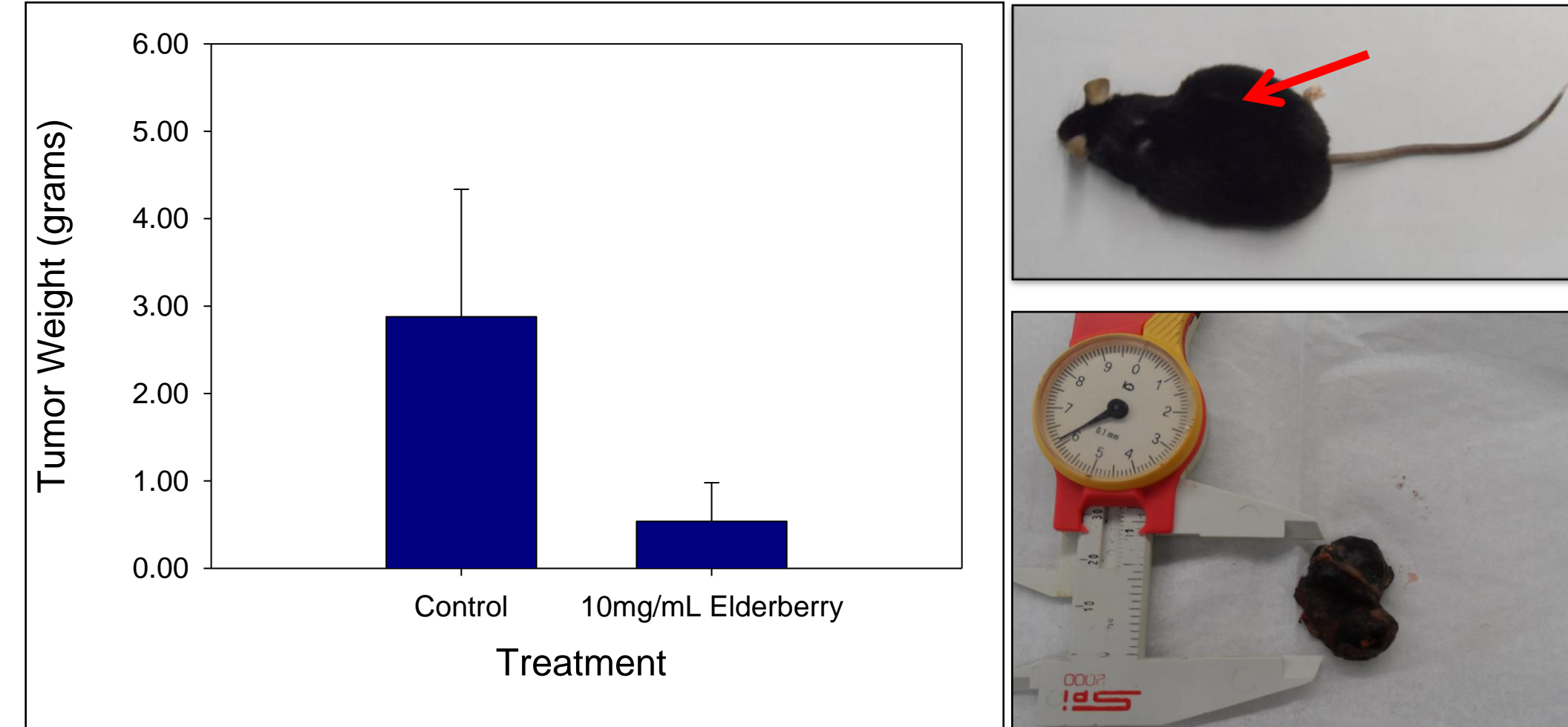


Figure 3. The Effect of Daily Elderberry Treatment on Melanoma Tumor Size. (Left Panel) Control and Elderberry treated mouse tumor weights after 14 days preventative treatments and 7 days post-cancer challenge treatments. (Upper Right Panel) A control mouse with a visible melanoma tumor. (Lower Right Panel) A metastatic tumor dissected from a control mouse.

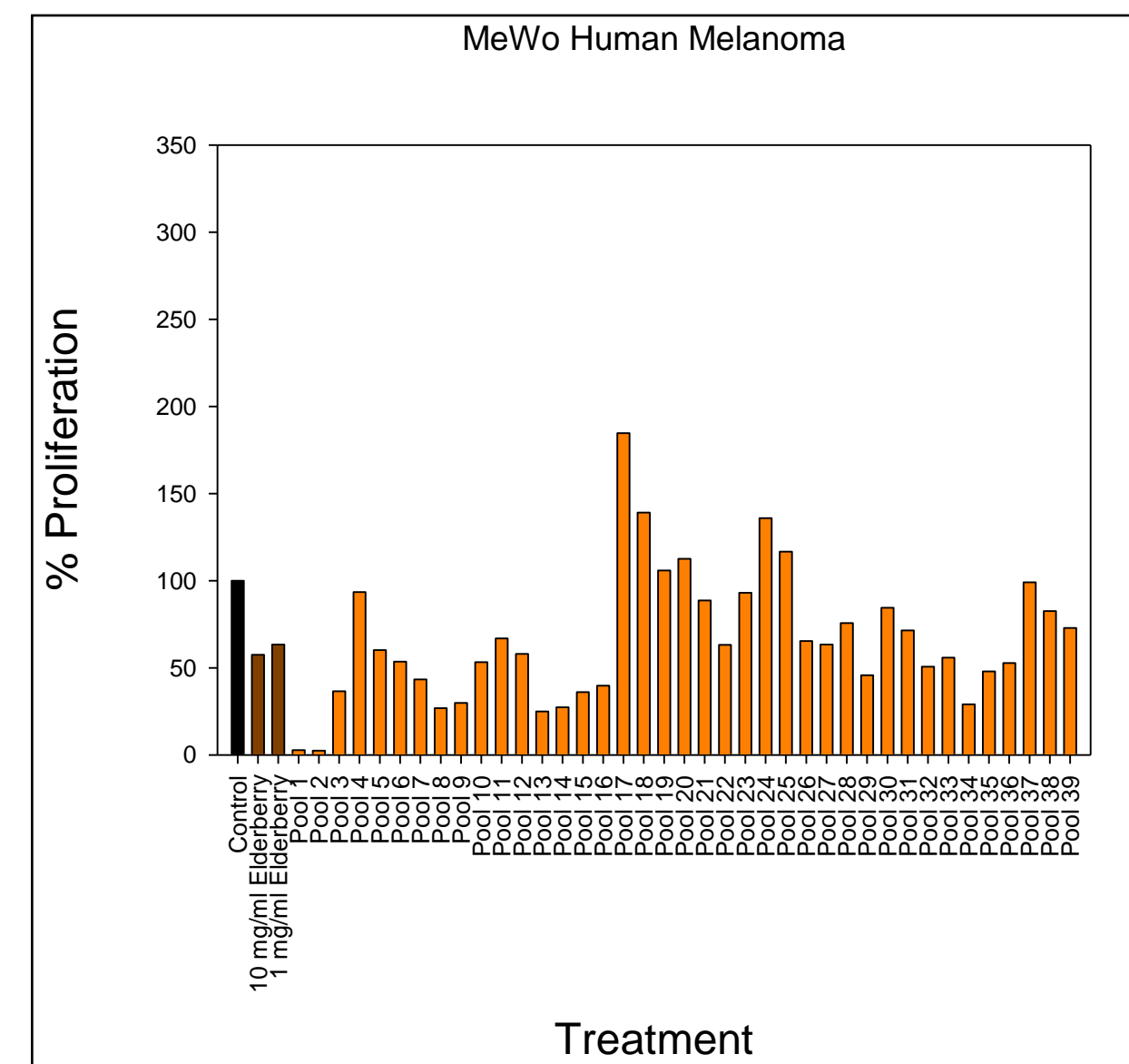


Figure 4. Proliferation Assay of Human Melanoma Cells. MeWo cells were treated with 10 μ L crude elderberry (10mg/mL and 1mg/mL) or pooled elderberry fraction. The percent proliferation of elderberry treated cells was based on growth of control cells at 100%.

¹ Youdin, K., Martin, A., and J. Joseph (2000). Incorporation of elderberry anthocyanins by endothelial cells increases protection against oxidative stress. *Free Radical Biology & Medicine*, 29(1): 51-60.
² Barak, V., Halperin, T., and I. Kalickman (2001). The effect of Sambucol, a black elderberry-based, natural product on the production of human cytokines: I. Inflammatory Cytokines. *European Cytokine Network*, 12(2): 290-296.

RESULTS

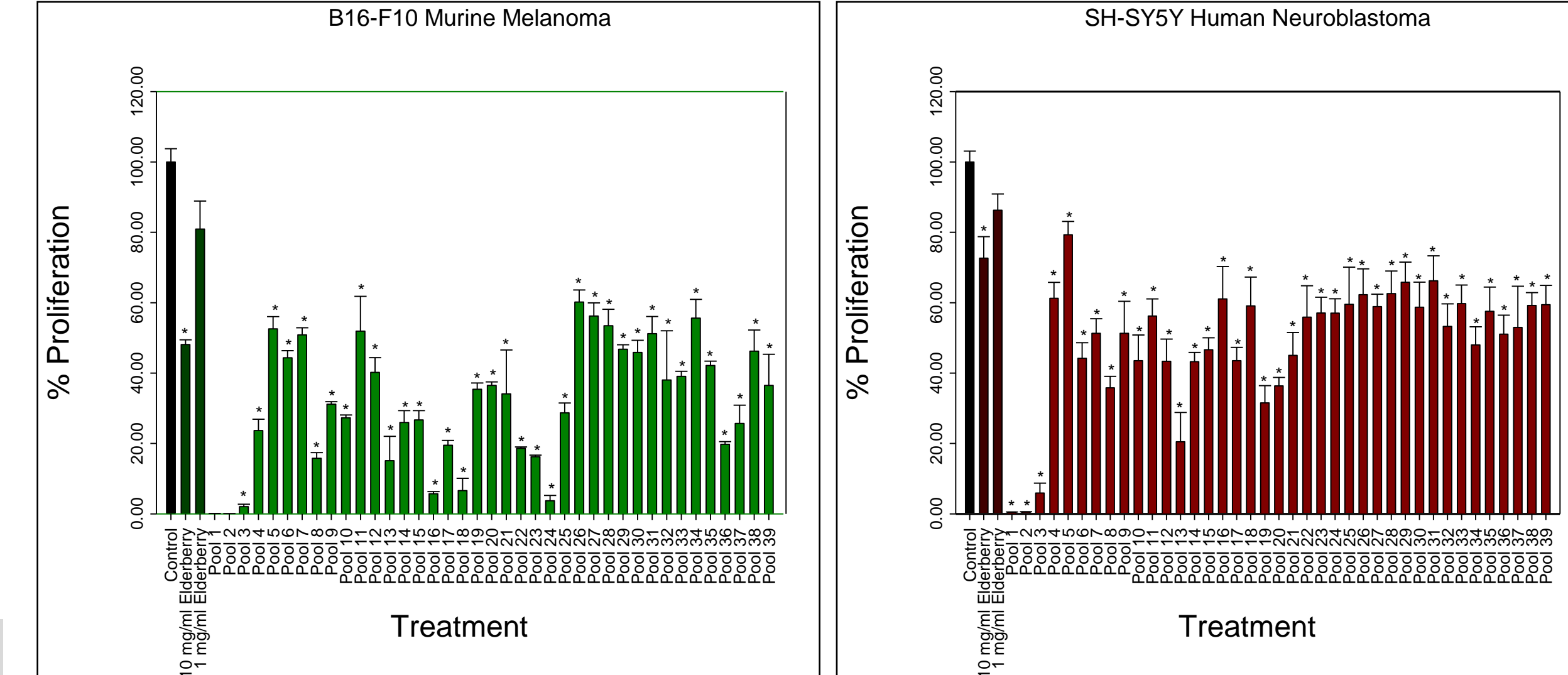


Figure 5. Proliferation Assay of Different Cancer Cell Lines. (Left Panel) B16-F10 cells and (Right Panel) SH-SY5Y cells were treated with 10 μ L crude elderberry (10mg/mL and 1mg/mL) or pooled elderberry fraction. The percent proliferation of elderberry treated cells was based on growth of control cells at 100%.

DISCUSSION

On average, control mice had larger tumors than elderberry-treated mice *in vivo* (2.88 g vs. 0.54 g, respectively).

- 67% of control tumors metastasized (vs. 0% elderberry-treated)

Pooled elderberry fractions significantly (*) decreased proliferation of multiple cancer cell lines *in vitro*.

- 72% of pooled fractions decreased MeWo growth by 20% or more
- 100% of pooled fractions decreased B16-F10 growth by 40% or more
- 100% of pooled fractions decreased SH-SY5Y growth by 20% or more

These results suggest that elderberry fractions may be useful in a naturopathic strategy to suppress multiple cancers and validates further study. Future experiments should aim to determine the chemical identity of active fractions and the cellular mechanisms by which they operate to inhibit tumor growth.

ACKNOWLEDGEMENTS

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