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# Investigating the therapeutic potential of capsaicin and curcumin: a comparative study on neuroblastoma and hypothalamic cells

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***Abstract*—Neuroblastoma is one of the most common malignant pediatric tumors. However, current cancer therapy usually involves surgical removal or chemotherapy, which are both damaging to normal cells. Phytochemicals are proposed as cancer treatment alternatives. Curcumin and capsaicin, two polyphenolic compounds from turmeric and pepper, possess anti-inflammatory and antioxidant properties, which suggest their neuroprotective benefits. This study investigates anti-cancer effects of curcumin and capsaicin on neuroblastoma cells in vitro while also probing potential effects of curcumin on hypothalamic neurogenesis in vivo. The experiments demonstrated that curcumin and capsaicin synergistically induced cell death, inhibited metastasis through decreasing MMP9 levels, and disrupted tumor growth of neuroblastoma cells. Both compounds also induced cell death of hypothalamic cells in vitro, and curcumin inhibited hypothalamic neurogenesis, possibly through the Notch signaling pathway. These results illustrate that while curcumin and capsaicin are effective at treating neuroblastoma cells, their toxicity on hypothalamic cells in vitro and potential impacts on neurogenesis in vivo must also be considered in conjunction with their anti-cancer effects in future research.**

## I. INTRODUCTION

Neuroblastoma, a malignant pediatric tumor in the sympathetic nervous system, is the most common cancer in infants in their first year[1]. The 5-year survival rate is only 40% for children with stage-4 metastatic neuroblastoma [2]. Currently, the most predominant cancer treatment involves surgical or radiation-based tumor removal and chemotherapy, which can have serious toxic effects on non-targeted tissues. Hence, scientists started exploring phytochemicals' anti-cancer potential, which are generally non-toxic, inexpensive, and can be found in common household spices.

Curcumin, a polyphenolic compound extracted from turmeric, and capsaicin, a primary capsinoid extracted from peppers of the Capscum genus, are known for their antioxidant and anti-inflammatory properties. On normal stem cells, curcumin can increase hippocampal neurogenesis in adult mice [3]. In colorectal cancer cells, curcumin decreased the survival rate of the cancer cells and inhibited metastasis through a downregulation of the matrix metalloproteinases gene group (MMP) [4]. Capsaicin acts as a cancer suppressor or promoter in different cancer cell types [5]. However, the combined effect of curcumin and capsaicin on neuroblastoma cells remains unknown.

## II. METHODS

MTT cell viability test was done on human neuroblastoma cells SK-N-SH and rat hypothalamus cell line R9 to detect reduction of MTT by mitochondrial dehydrogenase to a yellow formazan product after 24 hours of treatment. Cell viability was calculated as follows:

*Cell viability (%) = experimental OD value/control OD value × 100.*

The neuroblastoma cells' metastatic ability after treatment was analyzed through a sandwich enzyme-linked immunosorbent assay (ELISA) that detects the level of matrix metallopeptidase (MMP) -9 and a scratch-healing assay. Wound healing was assessed after 24 h. The percent decrease in migration was calculated as follows:

*Decrease in migration (%) = 1 − migration distance (pixels)/control migration distance (pixels) x100*

Neuroblastoma cell colony formation was also examined by measuring the percent area decrease of surviving colonies using ImageJ.

To investigate hypothalamic neurogenesis, transgenic larval zebrafish Tg(Tp1bglob:GFP), which expresses Notch-responsive cells, at 5 days post fertilization (dpf) were treated with curcumin. EdU immunohistochemical staining incorporates 5-ethynyl-2'-deoxyuri-dine (EdU) into the cells during the S phase of the cell cycle to express proliferating cells. The brains were dissected and imaged for the fluorescent EdU+ and TP1+ cells. Only cells in the posterior recess were counted.

## III. RESULTS AND DISCUSSION

### *Curcumin and capsaicin decreases neuroblastoma and hypothalamic cell viability*

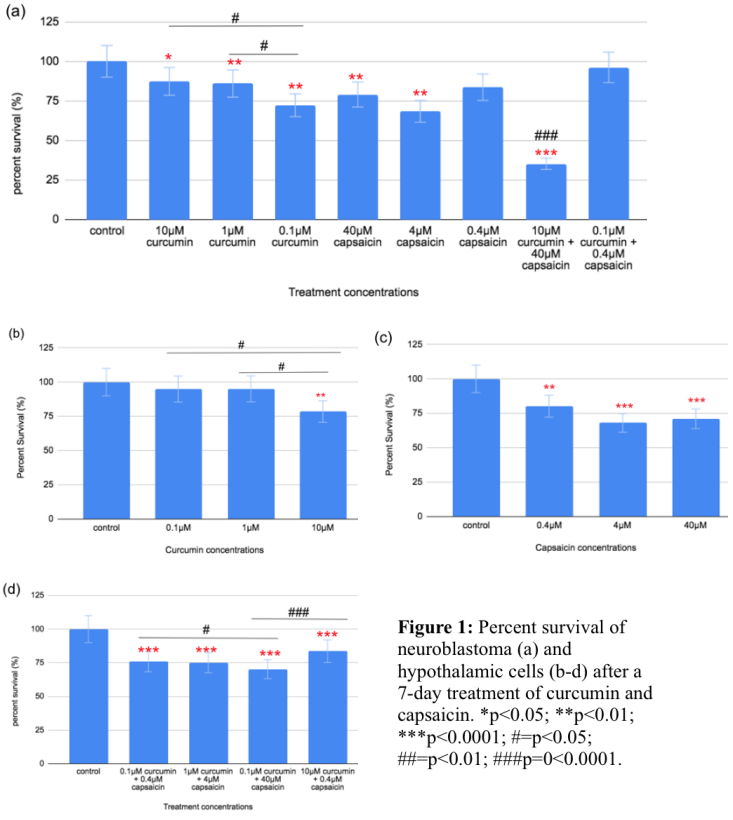
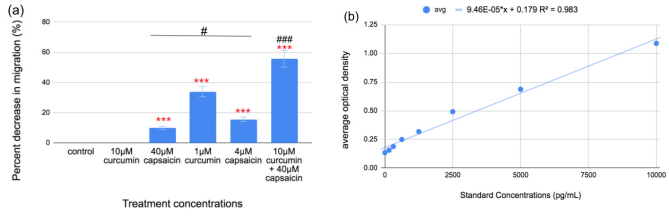



**Figure 1:** Percent survival of neuroblastoma (a) and hypothalamic cells (b-d) after a 7-day treatment of curcumin and capsaicin. *p<0.05; **p<0.01; ***p<0.0001; #=p<0.05; ##=p<0.01; ###p=0<0.0001.

### *Curcumin and capsaicin decreases neuroblastoma cell migration and MMP9 expression*



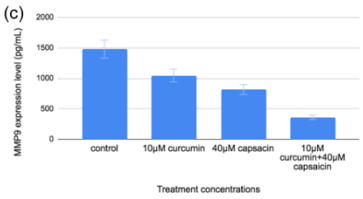



**Figure 2:** Effects of curcumin and capsaicin on neuroblastoma cell migration (a-b) and ELISA MMP9 level (c). *p<0.05; ***p<0.0001; #=p<0.05; ##=p<0.01; ###p=0<0.0001.

*Curcumin and capsaicin decreases neuroblastoma colony formation*

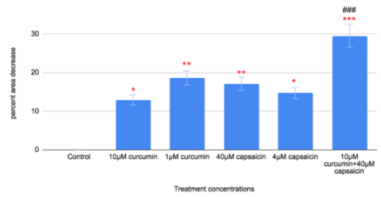



**Figure 3:** Effects of curcumin and capsaicin on the area of colonies. *p<0.05; **p<0.01; ***p<0.0001; #=p<0.05; ##=p<0.01; ###p=0<0.0001.

In this study, both curcumin and capsaicin decreased neuroblastoma cell viability and inhibited neuroblastoma cell metastasis, the invasion of cancer cells to neighboring cells which increases the tumor's risk level. An overexpression of MMP9, a protein involved in tumor invasion, metastasis and angiogenesis, has been examined in multiple malignant tumors such lung, pancreatic, breast cancers, etc. [7]. The results from the ELISA assay shows that curcumin and capsaicin reduced MMP9 expression level, indicating both chemicals inhibit metastasis through suppressing MMP9 expression. Curcumin and capsaicin also disrupted the tumor formation by reducing the area of neuroblastoma colonies. Normal cells accumulate in masses until they reach a finite density; however, cancer cells proliferate abnormally, resulting in high-density cell masses [8]. Prior studies of curcumin and capsaicin on other cancer cells are consistent with this study's findings that curcumin and capsaicin can interfere with tumor formation by disrupting the cell proliferation cycle, inhibit metastasis through regulating MMP9 level, and induce apoptosis [9] [10]. The most prominent anti-cancer effect occurred at the curcumin-capsaicin combination at 10μM and 40μM at all assays on neuroblastoma cells, indicating the two chemicals can synergistically suppress neuroblastoma growth (Fig 1, 2, 3).

*Curcumin decreases hypothalamic proliferating cells and Notch responsive cells*

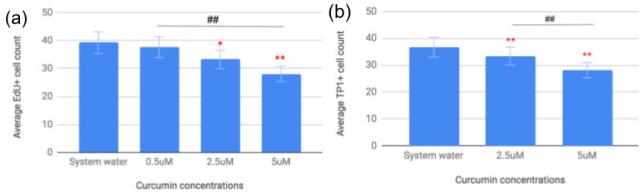



**Figure 4:** Effects of curcumin on (a) EdU+ (proliferating) cells and (b) TP1+ (Notch-responsive) cells. Boxed area indicates the posterior recess of the hypothalamus. *p<0.10; **p<0.05; ##p<0.05.

Curcumin and capsaicin decreased viability of hypothalamic cells, and that curcumin decreased cell proliferation in the postembryonic hypothalamus in zebrafish and reduced the number of Notch-signaling-responsive neural precursors in the hypothalamic stem cell niche (Fig 4). Notch is one of the three cell signaling pathways in zebrafish that regulates the neural stem cell cycle by inhibiting the differentiation of neural progenitor cells from oligodendrocyte precursors to oligodendrocytes and promoting the differentiation of glial precursors to astrocytes (Fig 5) [11]. According to a study done by Li et. al, blocked Notch signaling by DAPT, a γ-secretase inhibitor that indirectly inhibits Notch, leads to a significant decrease of proliferative cells from the control and the suppression of cell proliferation effects of curcumin in mice's hippocampus [12]. If considered together with Li et al's study, the results from this research may suggest that curcumin can block Notch signaling, which consequently triggers a positive feedback loop by decreasing proliferative cells and further inhibition of Notch signaling from curcumin.

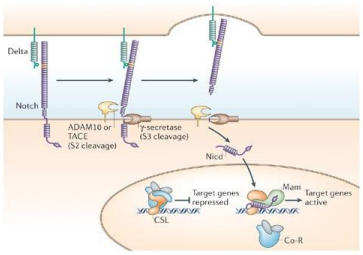



**Figure 5:** An Overview of Notch Signaling Pathway

## IV. CONCLUSION

While curcumin and capsaicin can be effective at treating neuroblastoma cells, they can decrease hypothalamic cell viability and postembryonic neurogenesis through the Notch signaling pathway, both of which are crucial for maintaining the body's homeostasis. By comparing the results of curcumin and capsaicin on neuroblastoma and hypothalamic cells holistically, this study provides insights on the clinical feasibility of curcumin and capsaicin on neuroblastoma treatment.

## V. ACKNOWLEDGEMENT

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