Proliferation evaluation using MTS assay on both cell lines treated with graduated doses of Rhodiola extract both before and after cell plate adherence.

**Methods:**
Two neuroblastoma cell lines, SK-N-AS (non-MYCN amplified) and NB-1691 (MYCN amplified), were evaluated. Cells were treated with graduated doses of *Rhodiola crenulata* extract pre and post plate adherence and results were compared to ethanol vehicle control and doxorubicin. Viability following treatment was evaluated using trypan blue exclusion and an MTS proliferation assay. Growth and migration was evaluated with a scratch wound assay.

**Results:**
Both NB-1691 and SK-N-AS cells treated with *Rhodiola* extract exhibited phenotypic changes within 24 hours following treatment. Upon *Rhodiola* exposure, cells became less adherent and exhibited reduced viability. These changes were enhanced upon treatment of cells prior to plate adherence (Figure 1,2). Further, cells treated with *Rhodiola* exhibited reduced cell growth and migration (Figure 4).

Following 72 hours of 200ug/ml *Rhodiola* treatment, 60% viability reduction of NB-1691 cells relative to vehicle control was observed (p<0.001). Cells treated with 10ug/ml doxorubicin exhibited a 13.7% viability reduction compared to control (p=0.04) after 72 hours (Figure 2). Proliferation of cells treated with 200ug/ml *Rhodiola* exhibited 25% reduction relative to control (p=0.001, Figure 3).

Treatment of SK-N-AS cells with 200ug/ml *Rhodiola* for 72 hours exhibited a 31.8% decrease in viability relative to vehicle control (p<0.001). Cells treated with 10ug/ml doxorubicin exhibited a 34.2% viability reduction relative to control (p=0.004) after 72 hours (Figure 2). Cells treated with 200ug/ml *Rhodiola* exhibited a 25% reduction in proliferation (p<0.001, Figure 3).

**Conclusions:**
*Rhodiola crenulata* extract was successful at causing cell death and reducing proliferation in both neuroblastoma cell lines. Its toxic effects were comparable to doxorubicin in the SK-N-AS line and were more pronounced in the chemo-resistant NB-1691 cell line. Future in vivo research is necessary to determine if *Rhodiola crenulata* extract will be an effective adjunct to standard multi-agent chemotherapy for the treatment of neuroblastoma.

**Purpose:**
The purpose of this study is to evaluate the effectiveness of a Tibetan plant extract, *Rhodiola crenulata*, as a treatment option for chemo-resistant neuroblastoma *in vitro*.

**Figure 1:**
Microscopic images of SK-N-AS and NB-1691 cells 24 hours following treatment with 200ug/ml *Rhodiola* extract or ethanol vehicle control. All cells were treated prior to plate adherence.

**Figure 2:**
Viability Analysis using trypan blue exclusion evaluating the cytotoxic effects of graduated doses of *Rhodiola* treatment in adhered and non-adhered cells of both cell lines. Results were compared to the cytotoxic effects of doxorubicin.

**Figure 3:**

**Figure 4:**
Scratch Wound Assay of SK-N-AS cells treated with *Rhodiola* vs ETOH vehicle control over 72 hours. Reduced growth was observed with cells treated with *Rhodiola*.

**Figure 3:**

**Figure 4:**

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