The anti-proliferative effect of turmeric and rooibos extracts on human Hodgkin lymphoma cells

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The Anti-proliferative Effects of Turmeric and Rooibos Extracts on Human Hodgkin Lymphoma Cells

Emily Kogel

March 29, 2016
Hodgkin Lymphoma

- Immune system support
- Lymph
  - Lymphocytes + other white blood cells
  - Antibodies
  - Nutrients
Apoptosis

Cancer Treatment: Options

- Surgery

Hodgkin Lymphoma:
- Radiation therapy
- Chemotherapy

http://www.joshgitalis.com/pre-and-post-surgery-nutrition/
http://www.dailymail.co.uk/health/article-1177248/New-scans-reveal-chemotherapy-working.html
Turmeric & Rooibos

- Natural health products - new cancer treatment options?

**Turmeric**
- Strong anti-inflammatory agent
- Supports immune system
- Activates intrinsic apoptosis

**Rooibos**
- Inhibitor of x-ray-induced cell transformation
- Anti-oxidant activity
- Free-radical scavengers, like flavonoids, induce apoptosis in cancerous cells

http://www.healingthebody.ca/healing-benefits-of-turmeric/
http://www.medicinehunter.com/sites/default/files/rooibos_main.jpg?1332955889
Methods of Extraction

- 3 solvents
- Whole, complex mixture

Hot Water

Cold Water

Ethanol
Do the **anti-proliferative properties** of turmeric and **rooibos extracts** in cancer cells translate to **Hodgkin lymphoma cells**, and if they do, is the mechanism of action **selective**?

**Diagram:**

- **Assess toxicity and efficacy *in vivo***
- **Discover the mode of action**
- **Determine selective cytotoxicity**
Do the anti-proliferative properties of turmeric and rooibos extracts in cancer cells translate to Hodgkin lymphoma cells, and if they do, is the mechanism of action selective?

**OBJECTIVES**

1. Determine if turmeric and rooibos extracts, both individually and in combination, exhibit cytotoxic effects against Hodgkin Lymphoma cells *(WST-1 assay)*

2. Quantify cytotoxicity *(Trypan blue exclusion assay)*

3. Determine permanency of the drugs’ effects *(Revival assay)*
1. Determining cytotoxicity

- WST-1 Assay

Lower absorbance = Lower viability
Cytotoxicity of Turmeric Extracts (KM-H2)
Cytotoxicity of Turmeric Extracts (KM-H2) - ROUND 2

**Cold Water**

- Concentration (mg/mL)
- Absorbance at 450 nm (% of Control)
- 48 Hours: Black bars, 96 Hours: Gray bars
- Significance: * indicates statistical significance

**Ethanol (Unfiltered)**

- Concentration (mg/mL)
- Absorbance at 450 nm (% of Control)
- 48 Hours: Black bars, 96 Hours: Gray bars
- Significance: * indicates statistical significance

Legend:
- 0.04
- 0.05
- 0.06
- 0.08
Cytotoxicity of Rooibos Extracts (KM-H2)

**Cold Water**

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Absorbance at 450 nm % of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>0.05</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>50</td>
</tr>
<tr>
<td>0.25</td>
<td>25</td>
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**Hot Water**

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<td>*</td>
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<td>0.75</td>
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* indicates statistical significance.
Cytotoxicity of Rooibos Extracts (KM-H2) – ROUND 2

Cold Water

Hot Water

Ethanol (Unfiltered)
2. **Quantify cytotoxicity**

- Trypan blue exclusion assay
2. Quantify cytotoxicity:

Effect of ethanolic turmeric extract on the viability of KM-H2 cells
2. Quantify cytotoxicity:

Effect of rooibos extracts on the viability of KM-H2 cells

Cold water rooibos

Hot water rooibos

Number of cells/mL

Time (hours)

Rooibos 0.1
Rooibos 0.2
Rooibos 0.3
VP16 (0.05)
Control
2. Quantify cytotoxicity:

Effect of extracts in combination on the viability of KM-H2 cells

Cold water combination

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Control</th>
<th>VP16 (0.05)</th>
<th>Turmeric EtOH 0.05</th>
<th>Rooibos 0.1</th>
<th>Turmeric EtOH 0.05 + Rooibos 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.0 \times 10^6</td>
<td>4.0 \times 10^6</td>
<td>6.0 \times 10^6</td>
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3. Determine permanency of the drugs’ effects

- Revival assay

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<th>48h</th>
<th>72h</th>
<th>96h</th>
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</thead>
<tbody>
<tr>
<td>Ineffective drug/Control</td>
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<tr>
<td>Semi-effective drug</td>
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<td><img src="image" alt="emoji" /></td>
<td><img src="image" alt="emoji" /></td>
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<tr>
<td>Effective drug</td>
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Growth of KM-H2 cells in various treatment groups:

PRELIMINARY RESULTS

48 hours (with drug)  
24-72 hours (without drug)
Conclusions

• Turmeric: ethanolic extracts exhibit anti-proliferative effects on Hodgkin lymphoma cells that are likely long-lasting

• Rooibos: hot water extracts show cytotoxicity against Hodgkin lymphoma cells

• A combinatorial effect exists when low doses of ethanolic turmeric and cold/hot water rooibos extracts are used together

These natural health products show potential to be studied further for the development of more effective chemotherapeutic agents.
Future Work

• Assess selectivity of extracts by studying cytotoxicity in normal lymphocytes

• Evaluate the mode of cell death induction

• Assess toxicity and efficacy alone and in combination in animal models
Dedication

• Dedicated to the memory of Kevin Couvillon, who lost his battle against Leukaemia in 2010.
Acknowledgments

- Dr. Pandey
- Members of Dr. Pandey’s Lab
- Funding
Thank you!