



# Effect of Polyphenolic Compounds on Apoptosis in an In-Vitro Model of Oxidative Stress

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## ➤ Background

- Oxidative Stress
- Oxidative Stress & Disease
- Catecholamines & Oxidative Stress
- Antioxidants as Therapeutics

## ➤ Objective

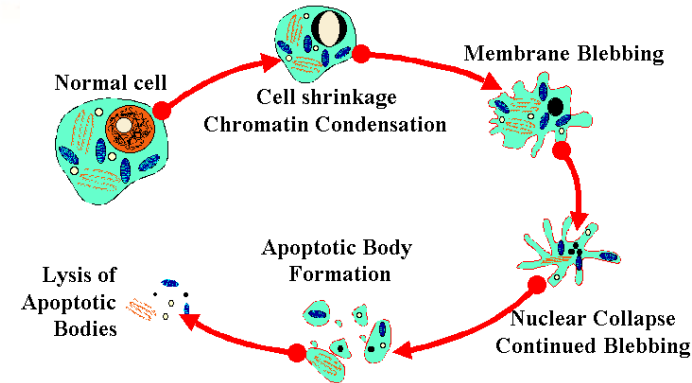
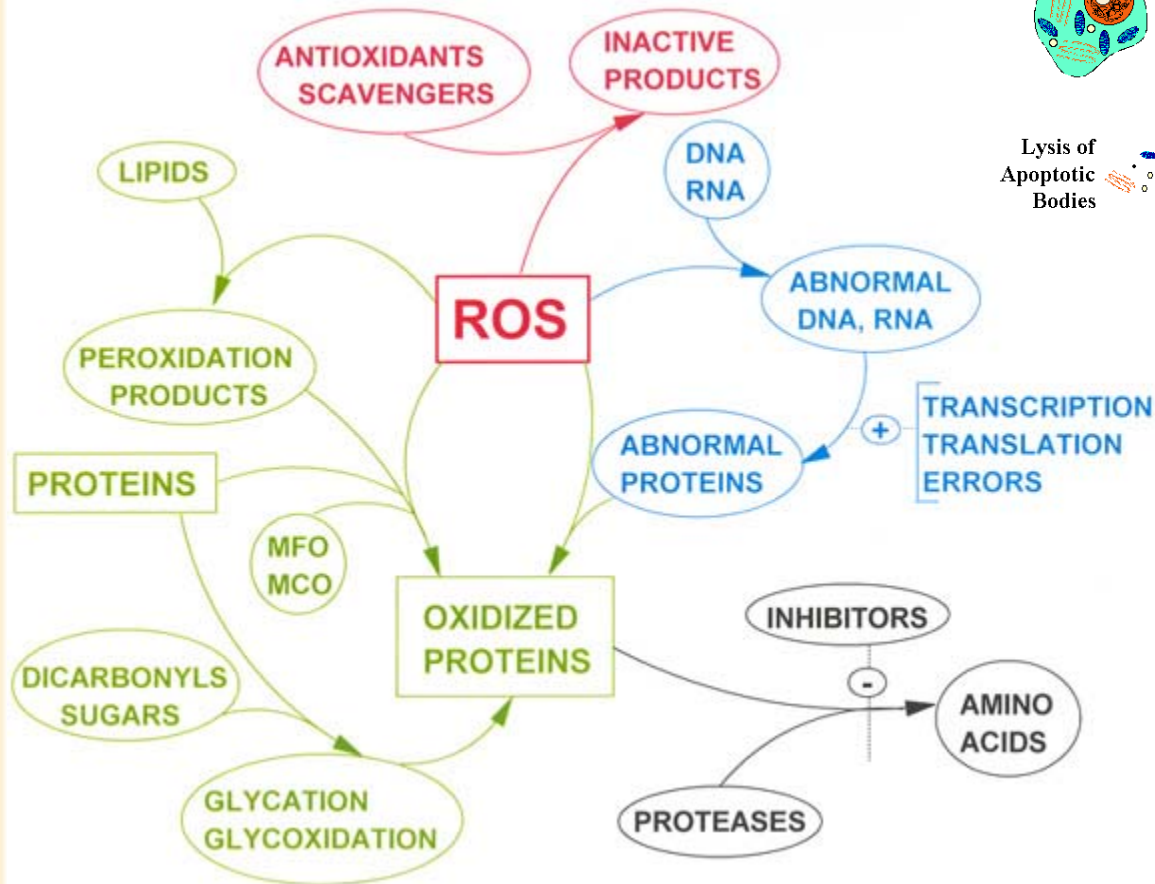
- Laboratory Techniques Used

## ➤ Results & Future Directions

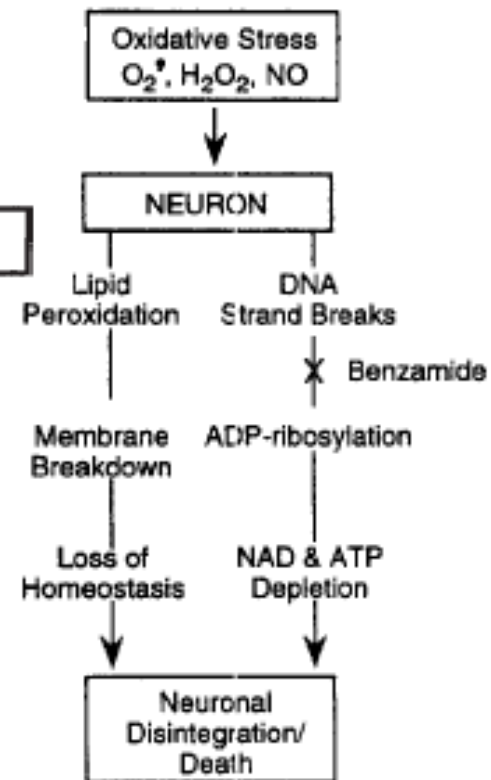
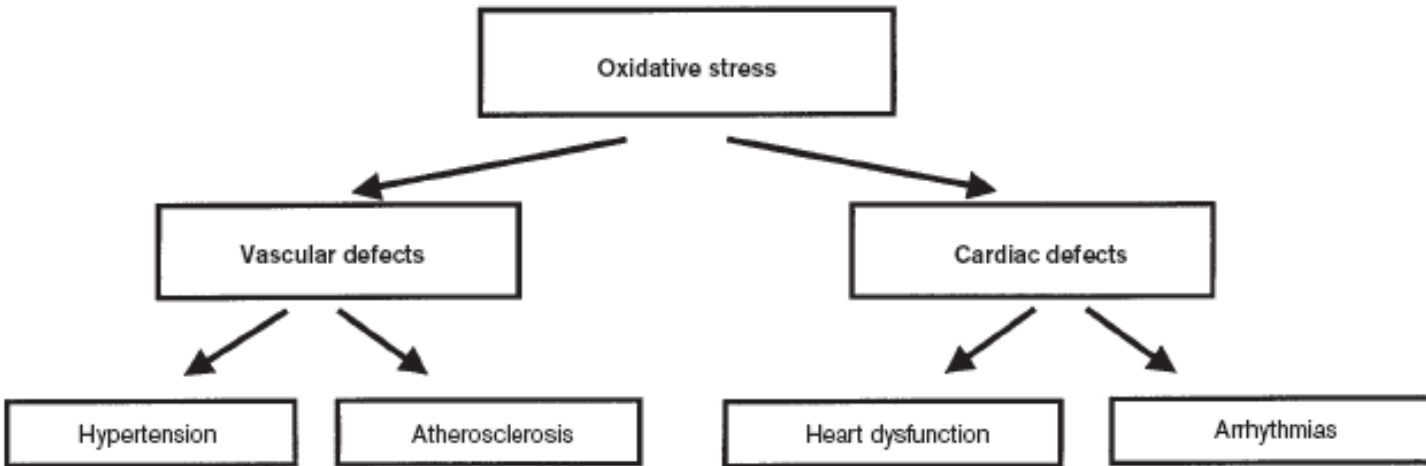
## ➤ Questions?



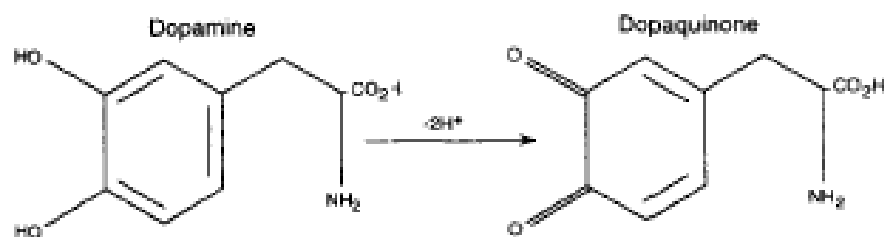
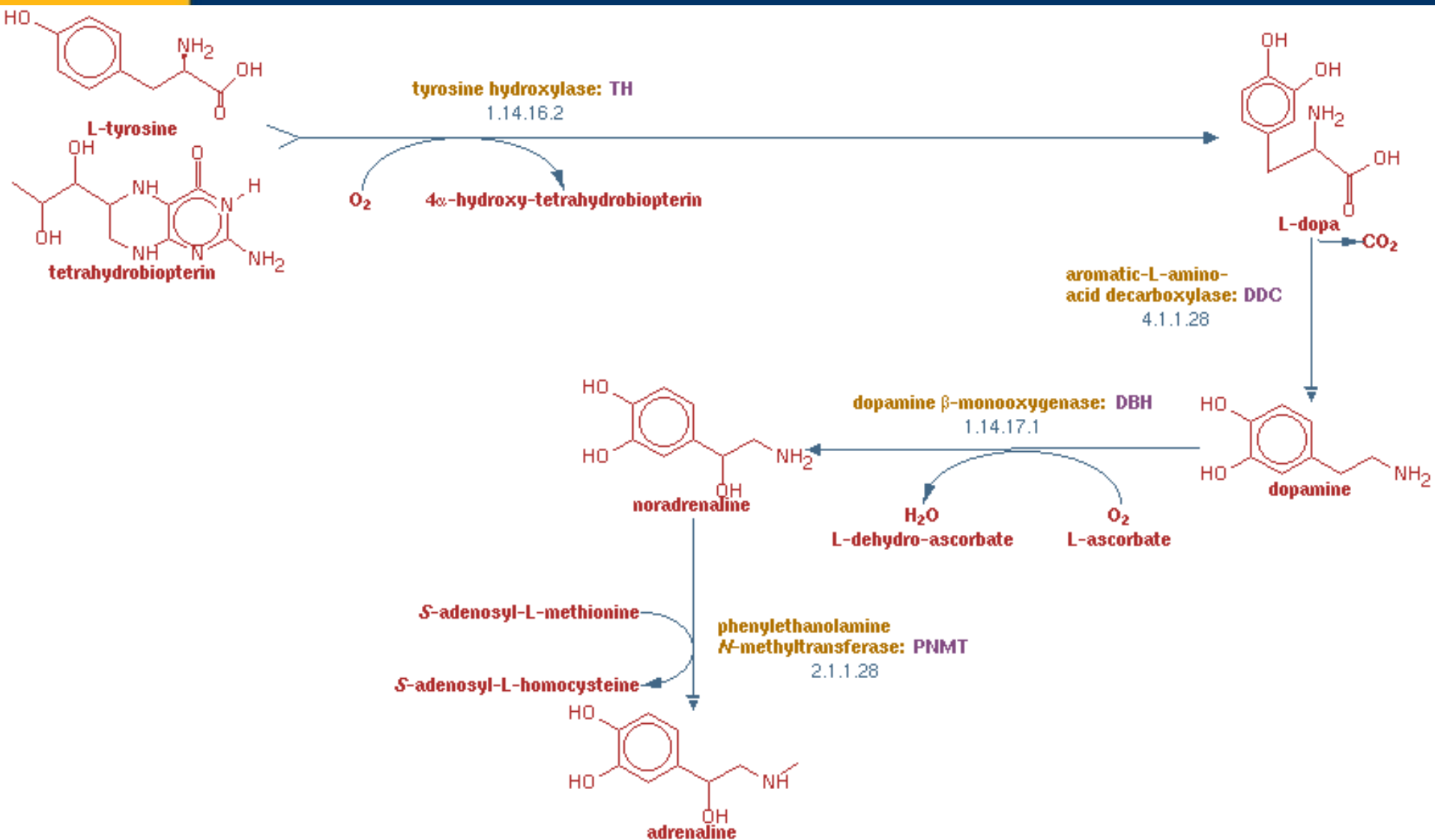
## Oxidative Stress



## Oxidative Stress & Disease



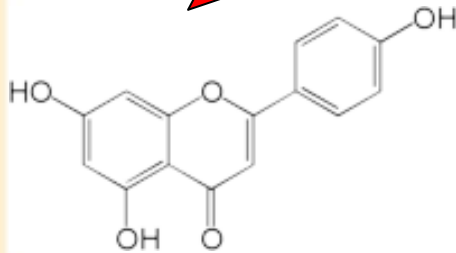
Previous research has found oxidative stress to be associated with hypertension / cardiac disorders, as well as neurodegenerative diseases.



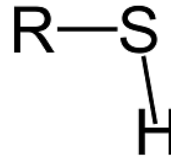
## Antioxidants as Therapeutics

Antioxidants are molecule capable of slowing or preventing the oxidation of other molecules.

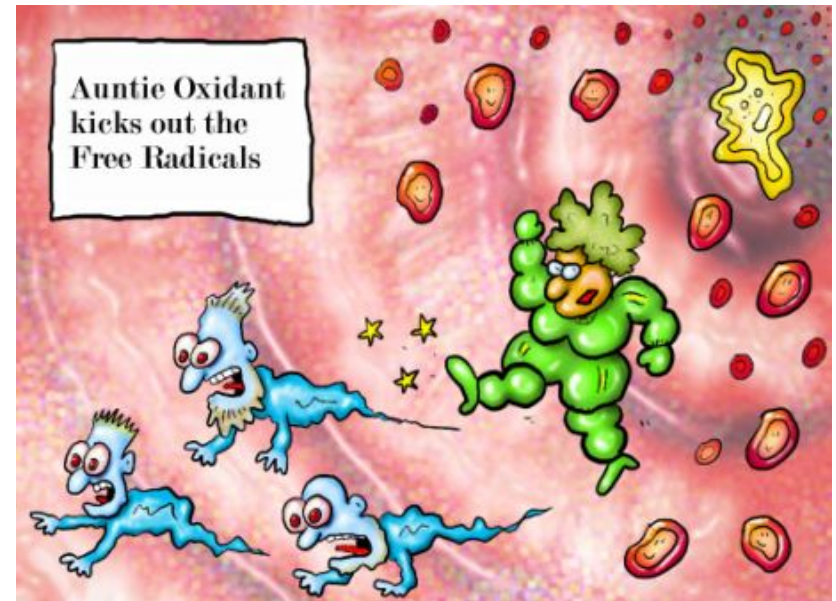
Reducing Agents



Polyphenols



Thiols



**Cardiovascular  
Health**

**Weight Loss**

**Neuroprotection**

**Antioxidants**

**Skin Damage**

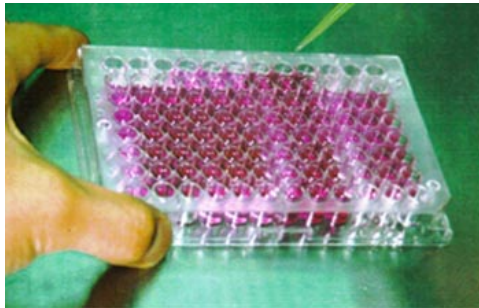
**Cancer  
Chemoprevention**

## Objective

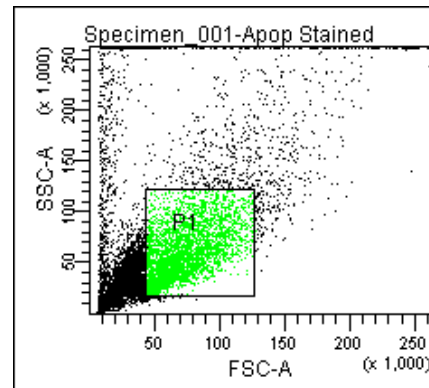
To examine the effect of polyphenolic compounds on cell viability in an *in-vitro* model of oxidative stress.



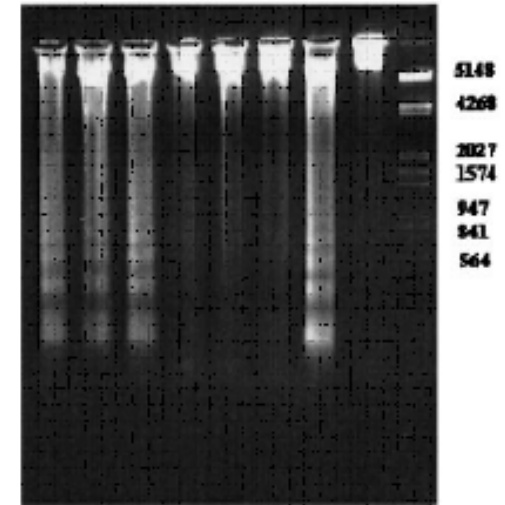
Northern Ontario  
School of Medicine



MTT Assay



Flow Cytometry

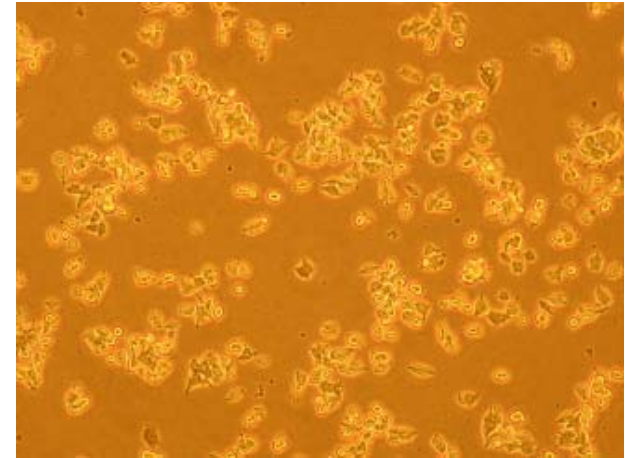
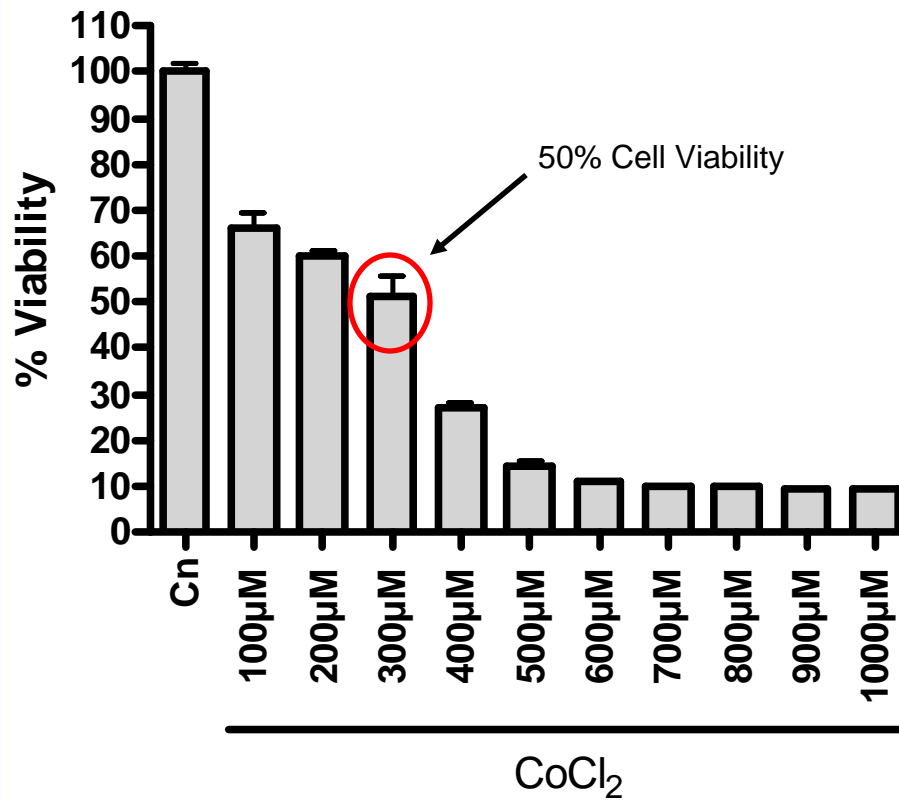


DNA Fragmentation

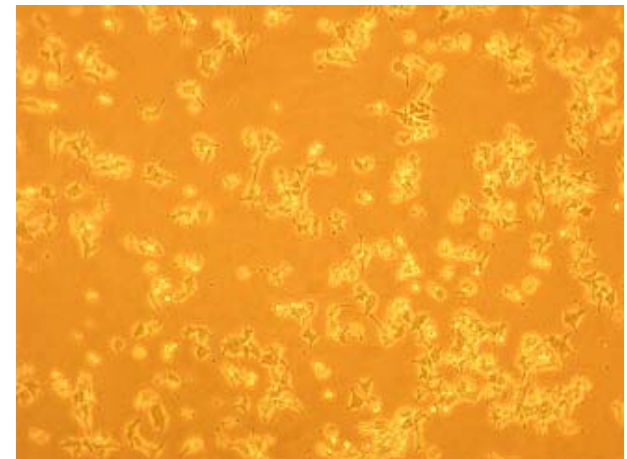


Caspase Activity

## MTT Assay - CoCl<sub>2</sub> Titration (Pass 20)



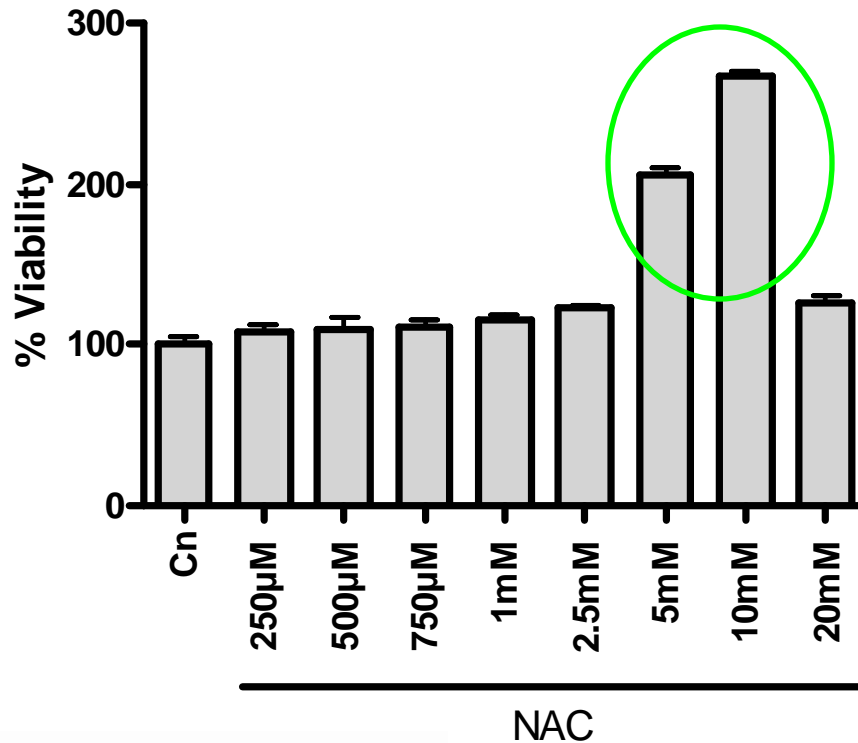
Control



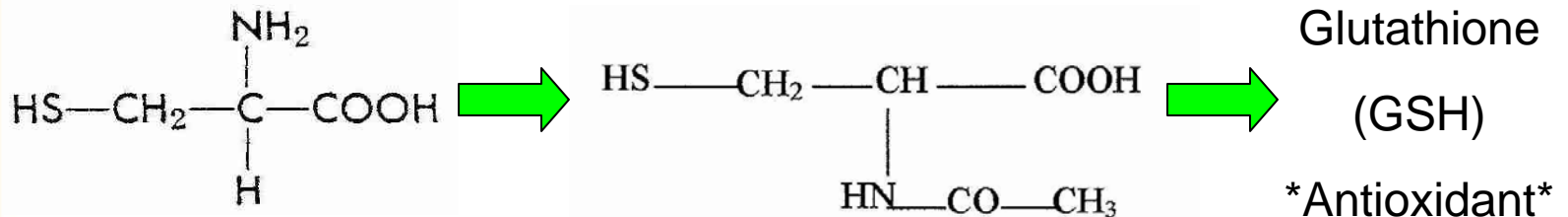
300µM CoCl<sub>2</sub>



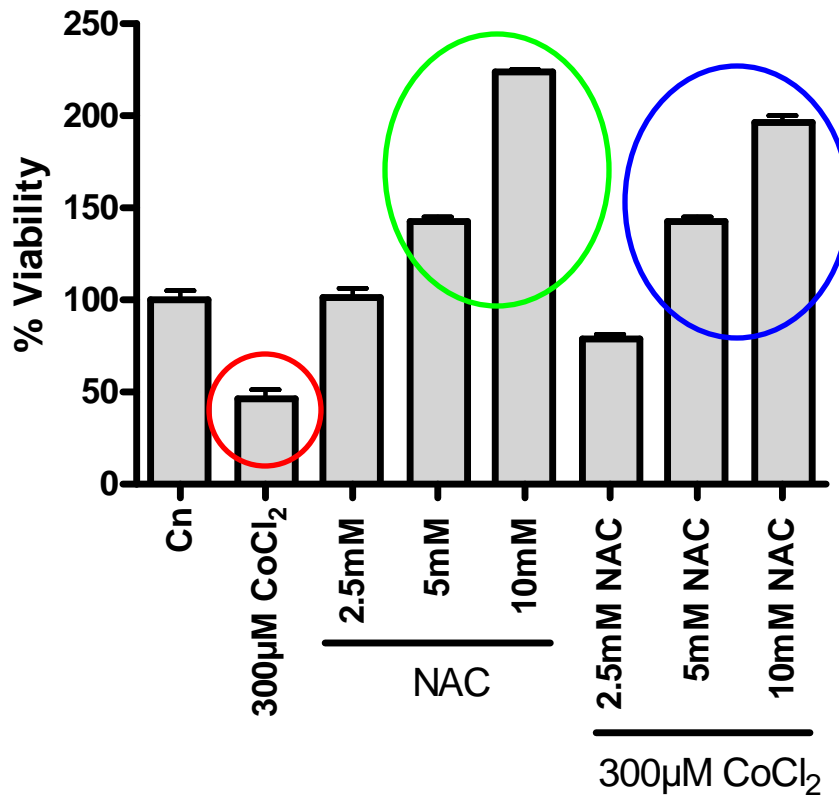
## MTT Assay - NAC Titration (Pass 22)



PC12 cells were treated with increasing concentrations of NAC (250µM-20mM) for 24h. Cell viability was then assessed via MTT assay.

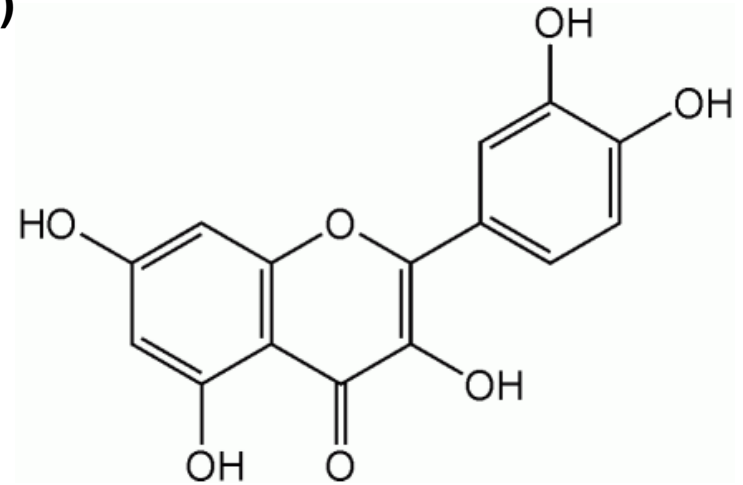
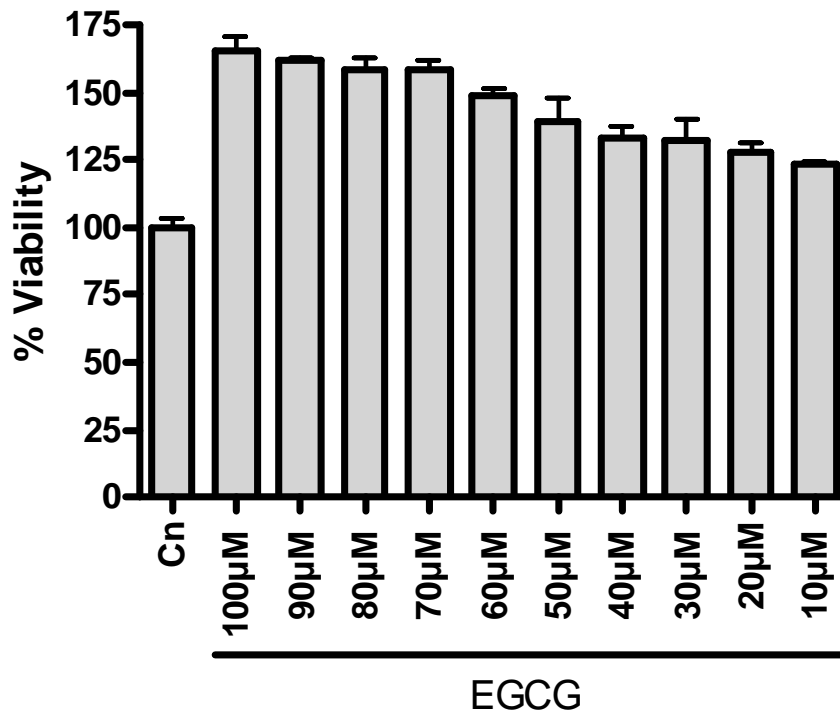


## MTT Assay - NAC Rescue (Pass 24)



PC12 cells were pretreated (30mins) with increasing concentrations of NAC (2.5, 5, 10mM) prior to exposure to CoCl<sub>2</sub> (300µM) for 24h. Cell viability was then assessed via MTT assay.

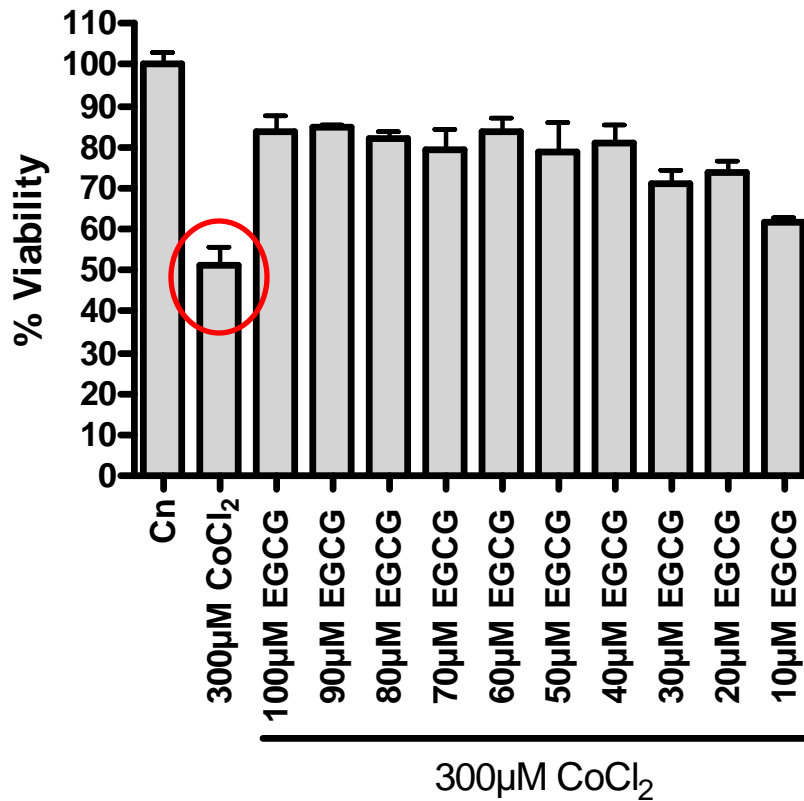
## MTT Assay - EGCG Titration (Pass 26)



### Epigallocatechin Gallate

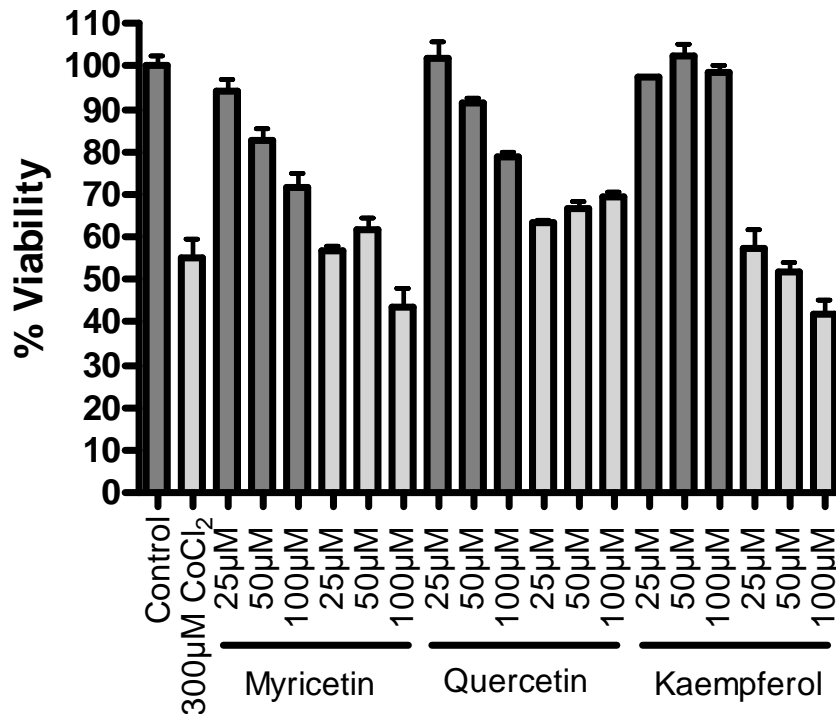
PC12 cells were treated with decreasing concentrations of EGCG (100µM-10µM) for 24h. Cell viability was then assessed via MTT assay.

## MTT Assay - EGCG Titration + CoCl<sub>2</sub> (Pass 25)

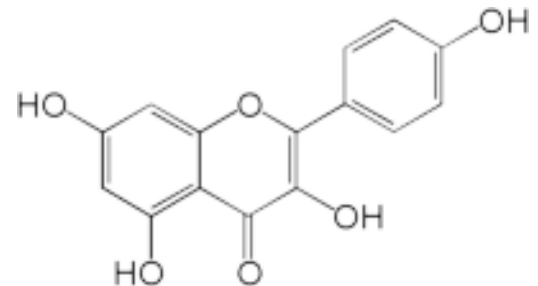
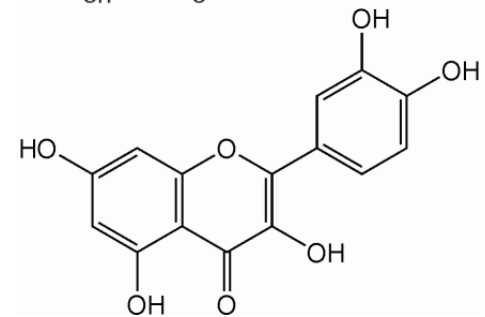
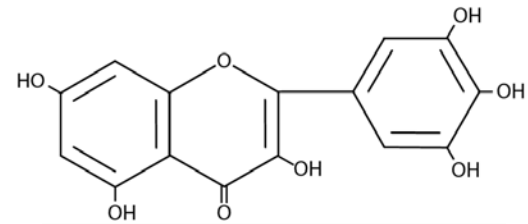


PC12 cells were pretreated (30mins) with decreasing concentrations of EGCG (100µM-10µM) prior to exposure to CoCl<sub>2</sub> (300µM) for 24h. Cell viability was then assessed via MTT assay.

## MTT Assay - Myricetin, Quercetin, & Kaempferol

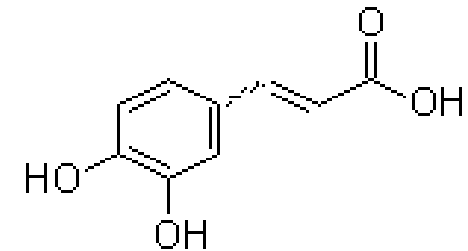
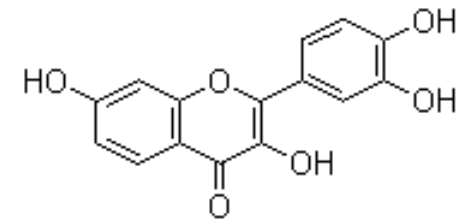
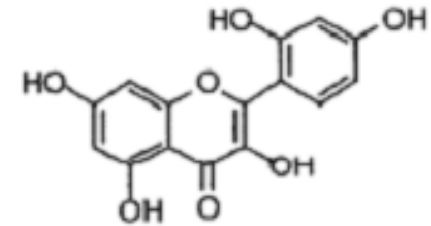
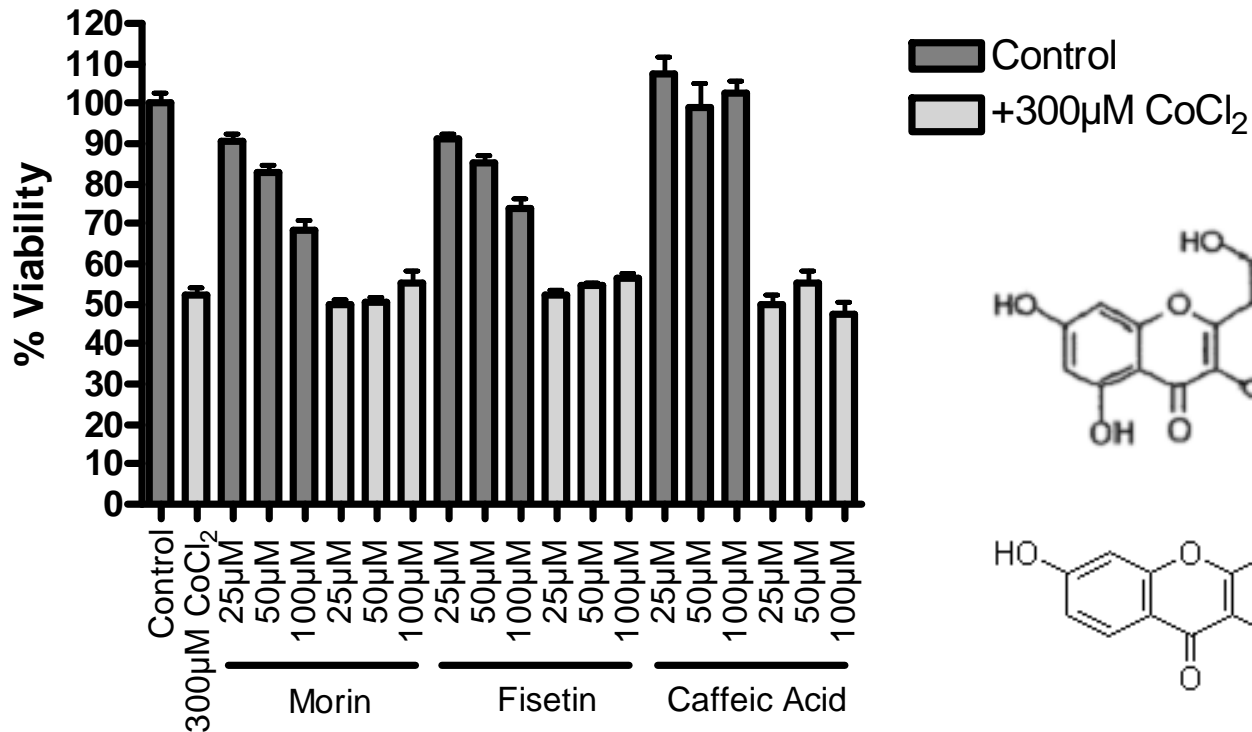


Control  
 +300µM CoCl<sub>2</sub>

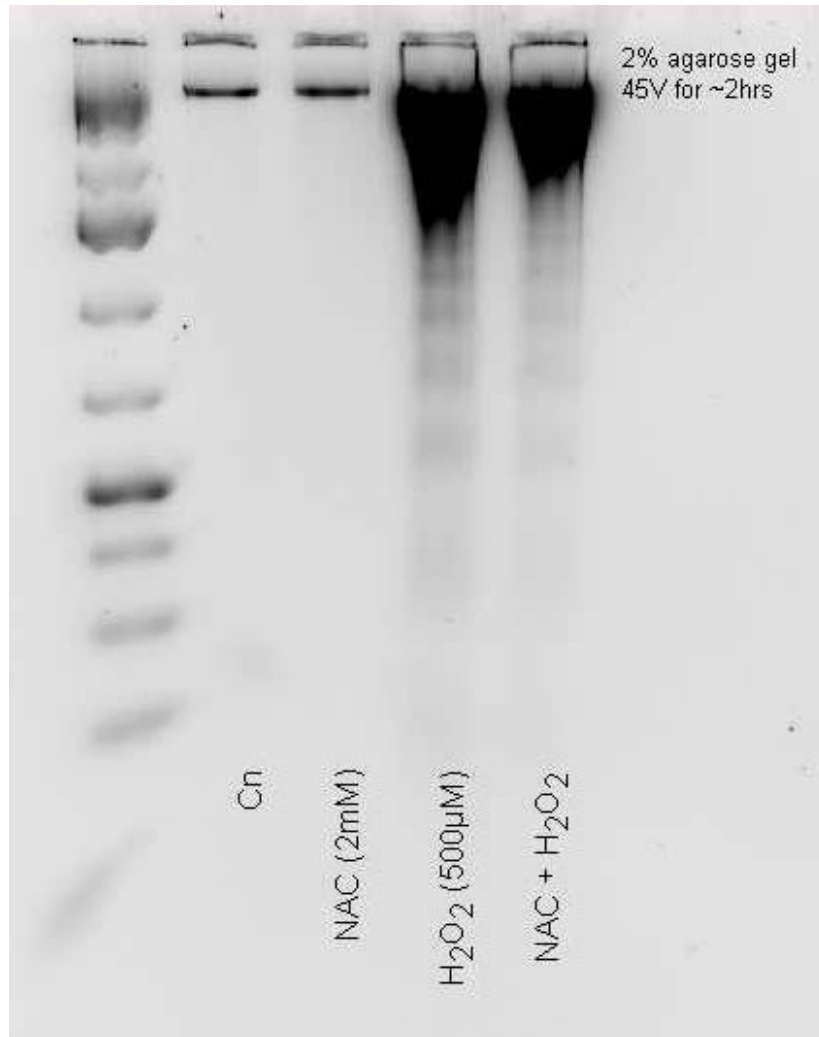


PC12 cells were pretreated (30mins) with polyphenols (25, 50, 100µM) prior to exposure to CoCl<sub>2</sub> (300µM) for 24h. Cell viability was then assessed via MTT assay.

## MTT Assay - Morin, Fisetin, & Caffeic Acid



PC12 cells were pretreated (30mins) with polyphenols (25, 50, 100µM) prior to exposure to CoCl<sub>2</sub> (300µM) for 24h. Cell viability was then assessed via MTT assay.



## DNA Fragmentation

PC12 cells were pretreated (30mins) with NAC (2mM) prior to exposure to H<sub>2</sub>O<sub>2</sub> (500μM) for 24h. Genomic DNA was then isolated and analyzed via gel electrophoresis.

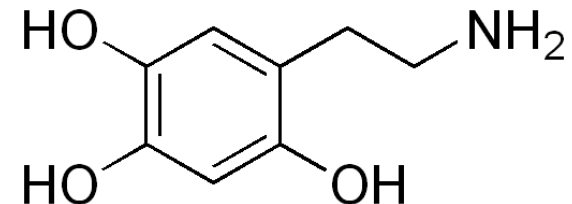
## Future Directions

Examine the effect of polyphenolic compounds on catecholamine synthesis, degradation, and secretion in an *in-vitro* model of oxidative stress.

### Explore Other Polyphenolic Compounds:

- Chlorogenic Acid
- Gallic Acid
- Methyl Gallate
- m-coumaric acid
- p-coumaric acid

Investigate effect of compounds on  $H_2O_2$  and 6-OHDA induced stress.



## Acknowledgements

### Supervisors:

Dr. T.C Tai

Dr. G.M Ross

### Labmates:

Gino Ubriaco – M.Sc. Candidate

Dominique Ansell – M.Sc. Candidate

Joe Eibl – Ph.D. Candidate

