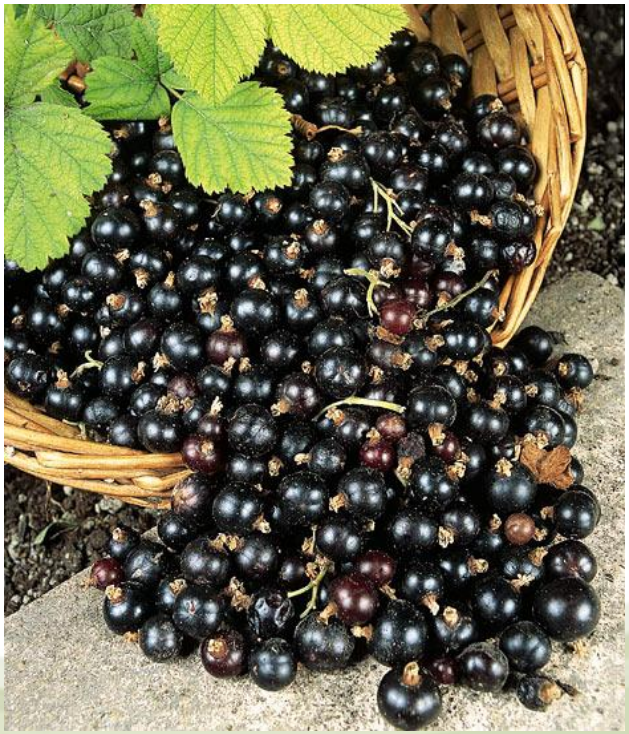
What are we up to with blackcurrant and human health?

Derek Stewart
Enhancing Crop Productivity and Utilisation



Food security has been at the forefront of many agendas.

Matched with this is economic stability and growth.

However health via dietary means has been busier that ever.

For blackcurrant, a minor crop in global terms, there have been several interesting advances with respect to human health

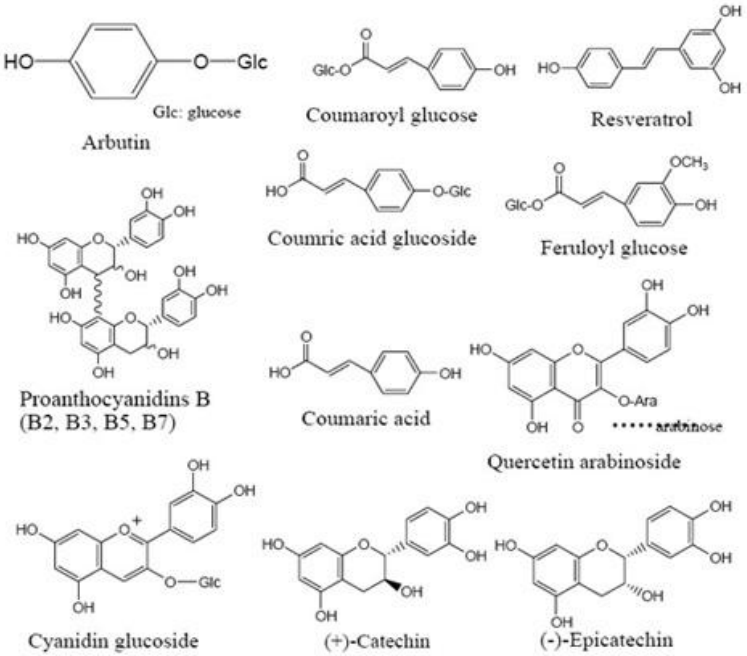
Berries…

- Good nutritional value
- Excellent source of bioactive compounds called polyphenols; Health beneficial effects
- Good source of antioxidants, compounds that *in vitro* are able to scavenge free radicals cause of cell aging and death… However….!!
- Is this what happens in vivo??

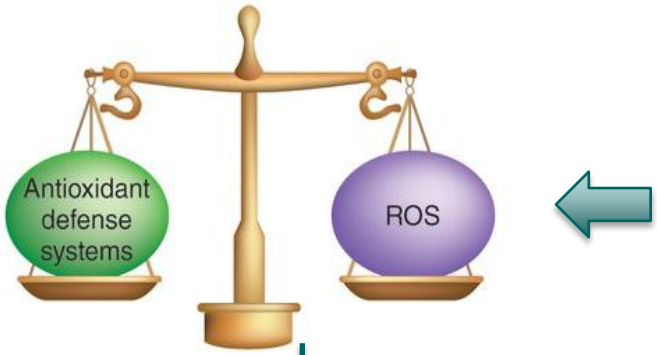
Polyphenols - bioactive compounds

- Polyphenols represent a group of secondary metabolites commonly found in higher plants
- Polyphenols are class of organic chemicals characterized by the presence of phenol structural units
- Due to the diverse biological properties <u>polyphenols are found to be potential candidates for use as a drugs to treat diseases such as:</u>

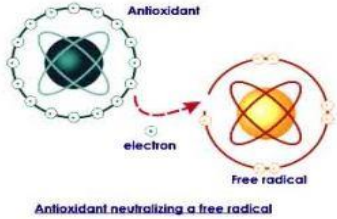
  Diabetes types I and II, cardiovascular diseases, cancer, AIDS, bacterial infections, neural disorders

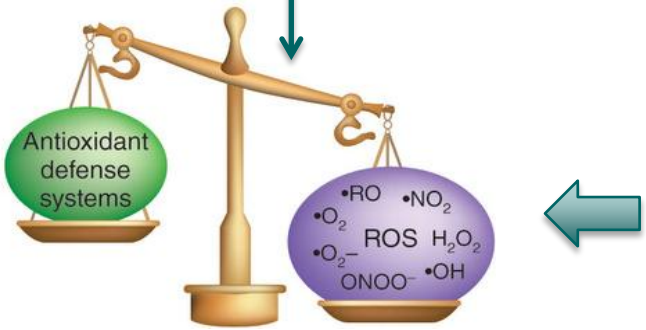


Oxidative stress couse of cell aging and death



Normal, balanced stage





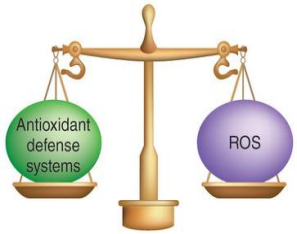
Source of Reactive Oxygen Species

- Air pollution
- UV rays
- Bio products from food and metabolism of chemicals
- Cellular respiration

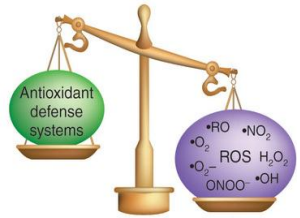
- Biomembrane damage
- DNA damage
- Protein damage
- Lipid peroxidation

Cancer CVD, Diabetes type I II, Neurodegenerative diseases

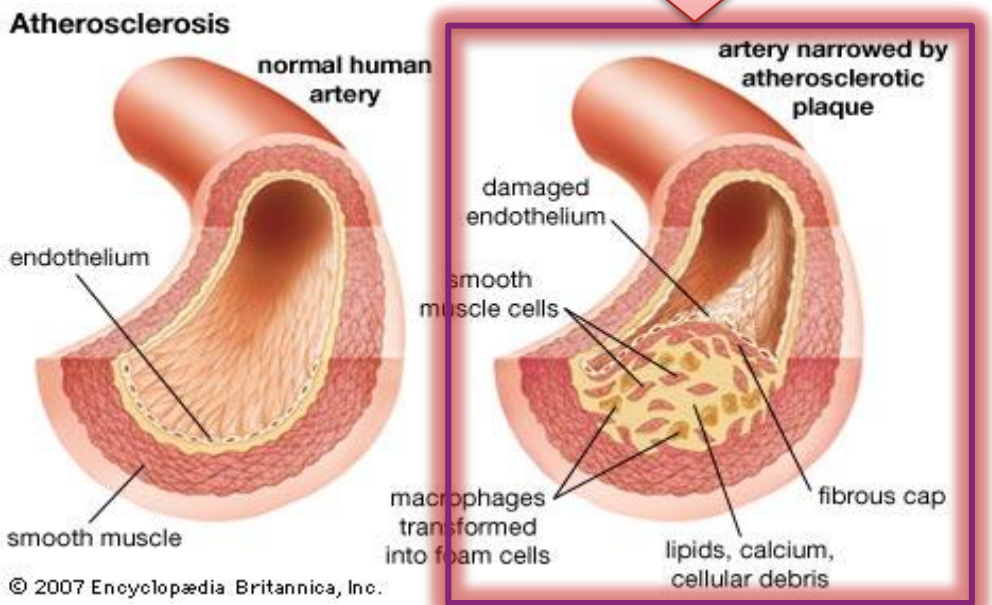
Free radicals cause cardiovascular diseases development



Oxidative stress



Oxidative stress



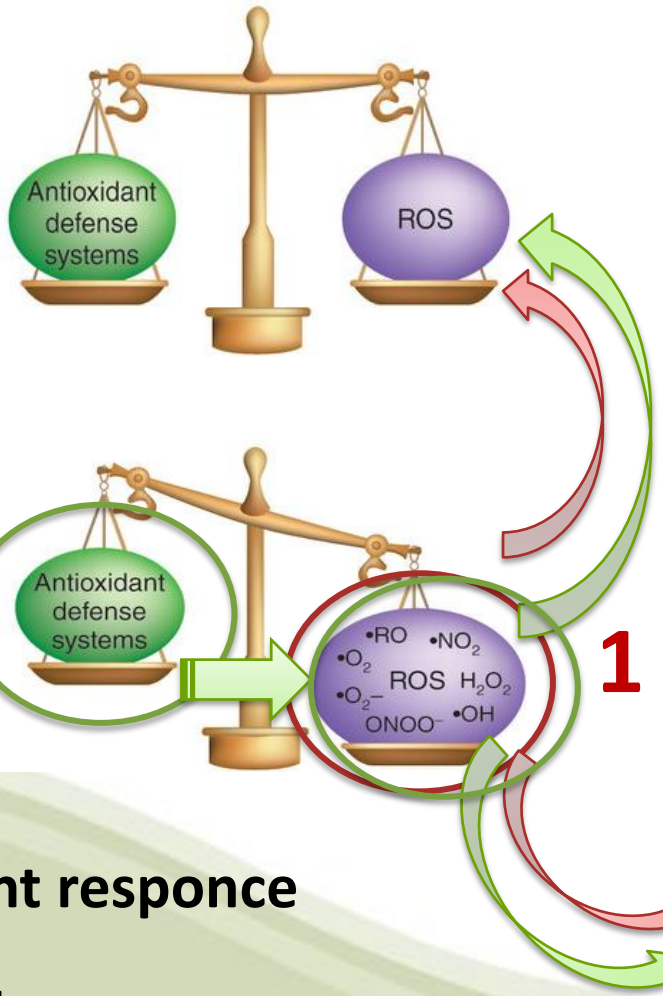
- **Protein damage and lipid peroxidation leads to loss of the membrane integrity, cell damage and death**
- **Biomembrane damage leads to the cell death**
- **DNA damage leads to mutations**

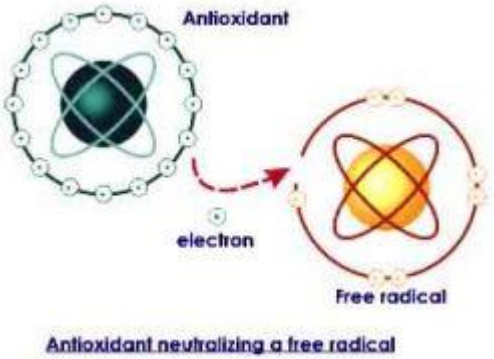
Stroke, Atherosclerosis,

High Blood Pressure, Heart attack

Possible role of polyphenols…

- **POLYPHENOLS can act through co-action with cell membrane receptors**
- **induction of the cell sygnalling pahways**
- **modulation of the genes and proteins expression**





1. Direct antioxidant responce

2. Indirect antioxidant responce

O_2, H_2O, NO_2 etc. Not harmfull to cells anymore

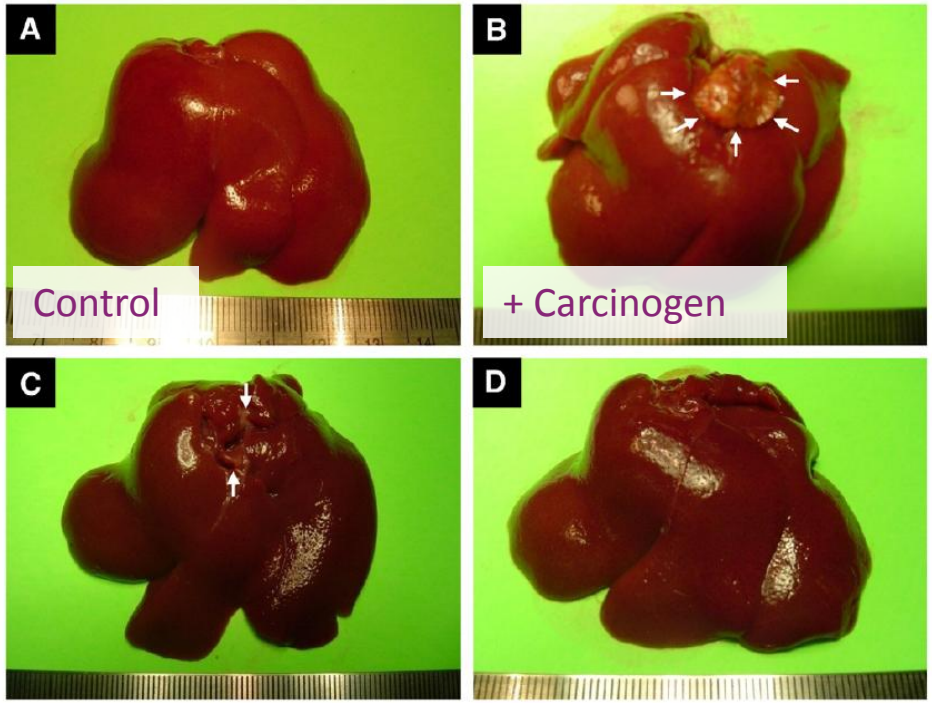
- So where are we with regard to health and blackcurrant?
- Vit C is still king and the way forward for health.
- However the “interesting stuff” is still derived from the polyphenols

Cancer

Reduced cancer levels in oxidant induced animal systems.

Cancer induction & promotion

± Blackcurrant polyphenol extract

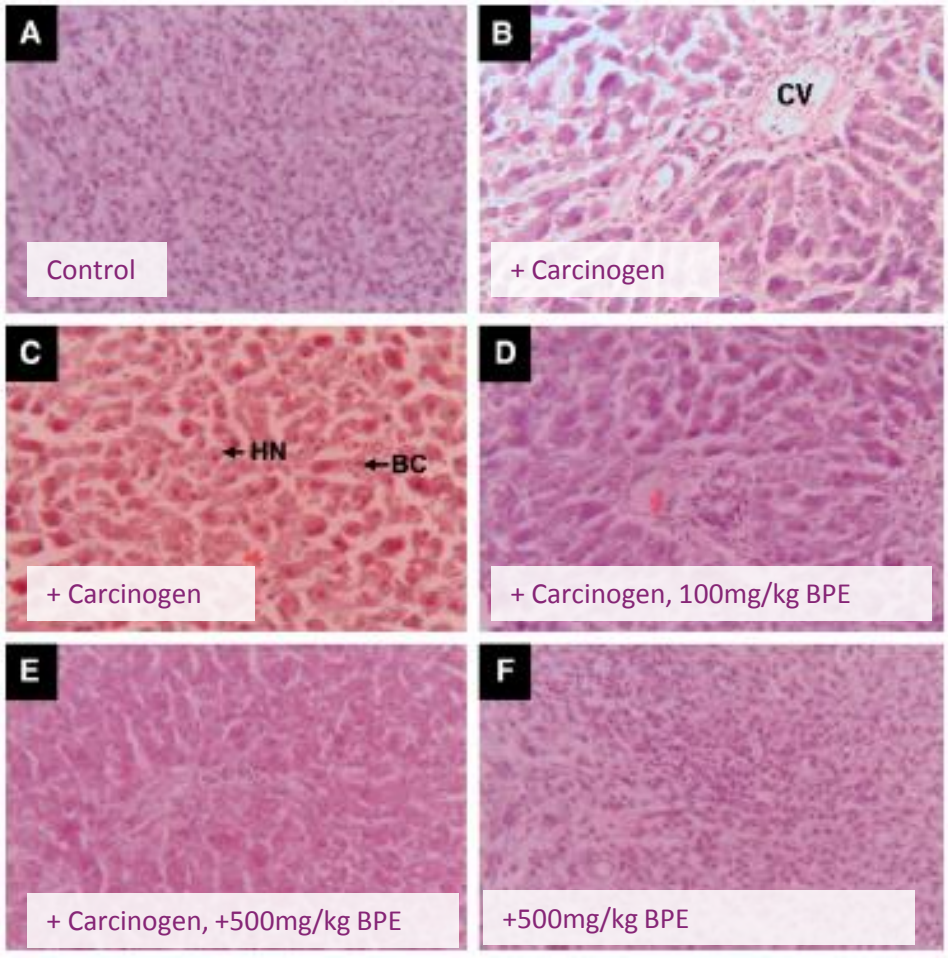


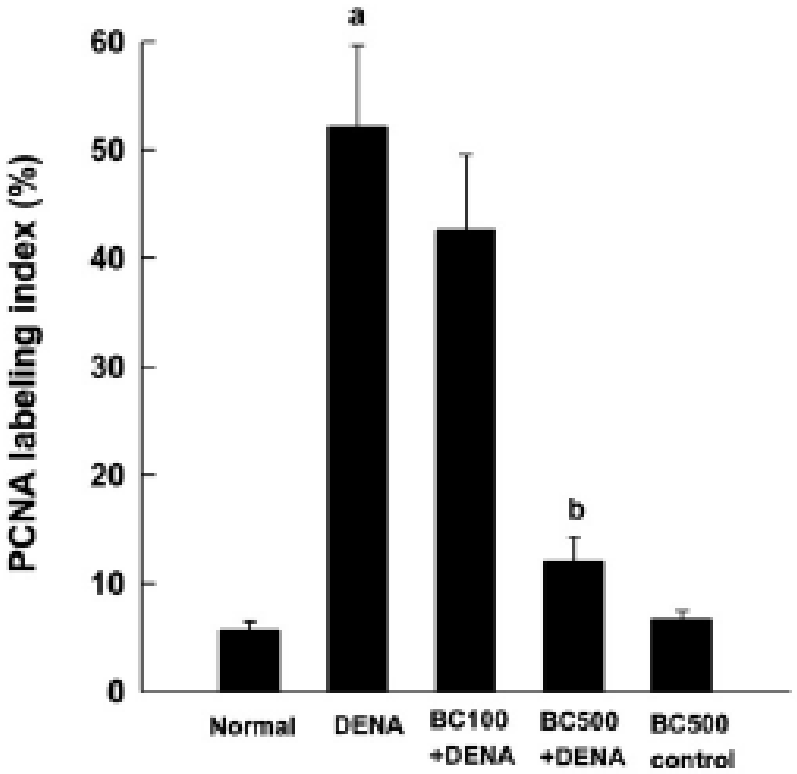
+ Carcinogen
+100mg/kg BPE

+ Carcinogen
+500mg/kg BPE

Reduction in macroscopic hepatocyte nodule number, size and proliferation.
BPE one had no deleterious effects

Bishayee (2011). J Nutrit. Biochem 22, 1035–1046.





Proliferative nuclear antigen (PCNA)

In Vitro (Cell) Systems

Black currant fraction	Cell line	Pharmacological effect	Reference
CARDIOVASCULAR SYSTEM			
Juice	J774A.1 macrophage cell line	Increased paraoxanase 1 expression, improving macrophage cholesterol attenuation	Rosenblat *et al.*, 2010[62]
Extract	Human umbilical vein endothelial cells	Increased activation of endothelial NO synthase and dilation of blood vessels	Edirisinghe *et al.*, 2011[69]
NERVOUS SYSTEM			
Extract	M1 transfected COS-7 cells	Increased recovery of calcium flux in type-1 muscarinic R's	Joseph *et al.*, 2004[70]
PULMONARY SYSTEM			
Extract (proanthocyanidin)	A549 alveolar epithelial cell line	Induced CCL26 secretion and amplified interferon-gamma	Hurst *et al.*, 2010[71]
TUMORS			
Whole fruit extract	HT29 colon cancer; MCF-7 breast cancer	Decreased the proliferation of cancer cells	Olsson *et al.*, 2004[72]
Whole fruit extract	HT29 colon cancer	Inhibited cancer cell growth	Wu *et al.*, 2007[73]
Whole fruit extract	HeLa cervical cancer	Reduced cell viability	McDougall *et al.*, 2008[74]
Juice	Caco-2 colorectal adenocarcinoma; MCF-7 and MDA-MB-231 breast cancer; AGS stomach adenocarcinoma; PC-3 prostate cancer	Suppressed cancer cell proliferation	Boivin *et al.*, 2007[75]
CAPS	Ehrlich ascites tumor	Exhibited cytotoxicity	Takata *et al.*, 2005[76]
Press residue extracts	Caco-2, HCT 116 and HT-29 colon cancer	Inhibited cell proliferation	Holtung *et al.*, 2011[77]
Skin extract	HepG2 liver cancer	Displayed antiproliferative effect	Bishayee *et al.*, 2010[78]

Gopalan (2012) Food and Function, In Press

Black currant fraction	Animal model **In Vivo: Model**	Pharmacological effect	Reference
CARDIOVASCULAR SYSTEM			
Oil (GLA)	Spontaneously hypertensive rats	Decreased blood pressure values	Engler, 1993[79]
Olive-blackcurrant-fish oil mixture	Wistar rats	Decreased serum TXA-B2 prothrombotic factor	Pregnolato, 1996[80]
Anthocyanin fraction	Sprague-Dawley rats	Decreased relative amount of hepatic saturated fatty acids and increased plasma tocopherol levels	Frank *et al.*, 2002[81]
Black currant concentrate	Norepinephrine-precontracted thoracic aortas of rats	Induced vasodilation via H1 receptors to increase NO levels	Nakamura et al., 2002[82]
Oil	Wistar rats	Inhibited accumulation of n-3 PUFA in liver and significantly decreased plasma GSH	Vecera *et al.*, 2003[83]
Seed oil	Wistar female rat blood samples	Decreased plasma GSH and *t*-butyl hydroperoxide-induced lipoperoxidation; did not effect hepatic GSH levels	Breinholt *et al.*, 2003[84]
Anthocyanin fraction	Watanabe heritable hyperlipidemic rabbits	Increased LDL and cholesterol and decreased VLDL content	Nielsen *et al.*, 2005[56]
Anthocyanin component (delphinidin-3-*O*-rutinoside)	rod outer-segment membranes in frogs	Inhibited endogenous NO and cGMP release	Matsumoto *et al.*, 2005[85]
Concentrate (delphinidin)	Sprague-Dawley rats	Decreased peripheral vascular resistance	Iwasaki-Kurashige *et al.*, 2006[86]

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NERVOUS SYSTEM			
Oil (GLA)	Streptozotocin-induced diabetes in mature Sprague-Dawley rats	Modulated TXA2 and increased motor nerve conduction velocity	Dines *et al.*, 1996[87]
OCULAR SYSTEM			
Juice extract (cyanidin)	Wistar rats and Japan White rabbits	Improved rhodopsin regeneration and dark adaptation by enhancing rhodopsin precursor formation	Matsumoto *et al.*, 2006[88]
Extract	1 day old white Leghorn chicks	Inhibited enlargement of the globe component dimensions in artificially induced myopia	Iida *et al.*, 2010[89]
PULMONARY SYSTEM			
Leaf extract (proanthocyanidin)	Saline-induced pleurisy and carrageenin-induced right hind limb edema in male Wistar rats	Decreased inflammation and inhibited neutrophilic cellular infiltration	Garbacki *et al.*, 2004[48]
SKELETAL SYSTEM			
Seed oil	Monosodium urate crystal-induced inflammation in subcutaneous air pouches formed in Sprague-Dawley rats	Inhibited formation of monosodium urate crystal formation	Tate *et al.*, 1994[90]
TUMORS			
Oil (GLA)	Metastatic 13762MAT:B breast tumor in the lungs of Fischer rats	Reduced the number of foci and tumor burden	Karmali *et al.*, 2004[91]
Juice	Xenografted Ehrlich ascites tumor in ICR mice	Inhibited tumor growth	Takata *et al.*, 2005[76]
Modified CAPS	Xenografted Ehrlich ascities tumor in ICR Mice	Reduced tumor weight	Takata *et al.*, 2007[92]
Skin extract	DENA-initiated and PB-promoted hepatocarcinogenesis in Sprague-Dawley rats	Suppressed the number, size, and volume of hepatocyte nodules	Bishayee *et al.*, 2011[93]
		Lowered the number and area of GGT-positive foci; reduced the expression of HSP70, HSP90, COX-2 and NF-κB	Bishayee *et al.*, 2012[94]
		Diminished lipid and protein oxidation; reduced the expression of iNOS; 3-NT, antioxidant enzymes and Nrf2	Thoppil *et al.*, 2012[95]

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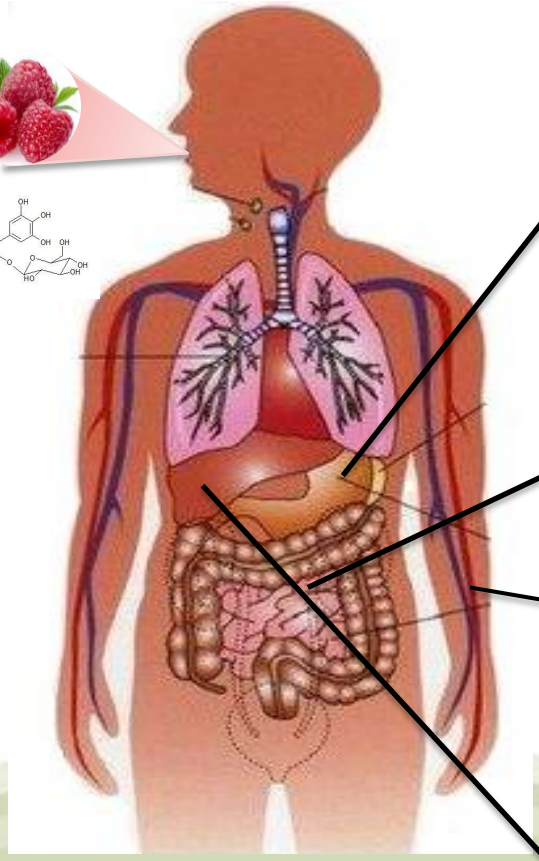
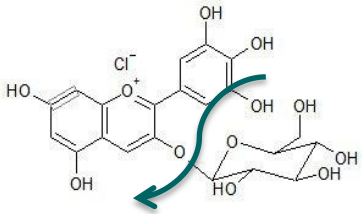
Black currant fraction	Clinical study	Clinical effect	Reference
CARDIOVASCULAR SYSTEM			
Seed oil (GLA)	23 cryptogenic ischemia stroke patients undergoing transesophageal echocardiography, 26 known-cause stroke patients, 57 non stroke controls	Inhibited platelet formation, decreased fibrin formation, and increased anti-coagulant effect	Stone *et al.*, 1995[96]
Concentrate (anthocyanin) ✓	Right trapezius muscles in 20 healthy human subjects	Induced relief of shoulder stiffness and decreased muscle fatigue via improved blood flow	Matsumoto *et al.*, 2005[97]
Oil	Randomized, double- blind, crossover study of 15 healthy female subjects administered black currant seed oil supplements	Decreased LDL cholesterol levels when administered with fish oil	Tahvonen *et al.*, 2005[98]
Juice ✓	Serum inflammatory markers in 48 peripheral artery disease patients	Reduced serum inflammatory markers such, e.g. C reactive protein	Dalgard *et al.*, 2009[99]
Seed press residue	Serum and stool tocopheral concentrations in 36 healthy female subjects	Increased alpha- and gamma-tocopherol serum concentrations	Helbig *et al.*, 2009[51]
Oil (soft capsule)	Observational study of 2154 dyslipidemic patients	Increased serum HDL-C protein and lowered triglyceride and total cholesterol in low BMI patients with hyperlipidemia	Fa-Lin *et al.*, 2010[100]
20% Juice (anthocyanin) ✓	Randomized, cross-over, double-blind, placebo-controlled acute meal study in 11 female and 9 male healthy volunteers	Did not have significant effect on total plasma nitrate, nitrite, ICAM, or VCAM levels	Jin *et al.*, 2011[101]
anthocyanin ✓	Cross-over study in 12 hypercholesterolemic patients	Increased NO-cGMP activation, improved serum lipid profile, decreased inflammatory markers	Zhu *et al.*, 2011[102]

Absorption and metabolism of delphinidin

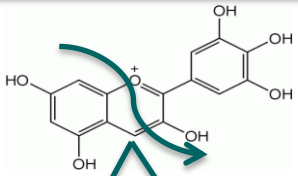


- GUT — pH ~ 4



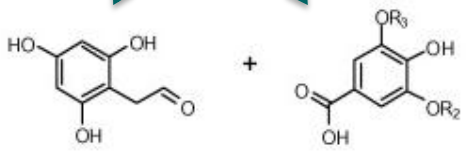
Delphinidin-3-*O*-glucoside

- Small intestine — pH ~ 7 - 8



Delphinidin

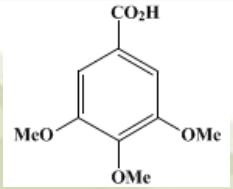
- Blood — pH ~ 7.4



aldehyde + phenolic acid

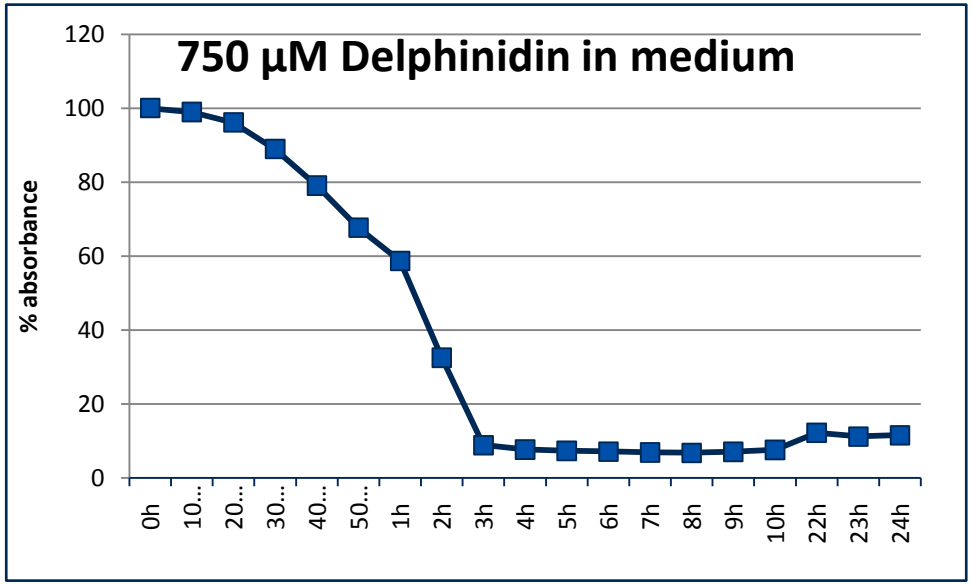
prologlucinaldehyde + gallic acid

- Liver



- Methylation
- Sulphation
- Glucuronidation

Delphinidin is very unstable compound



Anthocyanin stability is strongly affected many factors, such as:

- temperature
- solvents
- pH)
- anthocyanin structure
- light
- anthocyanin concentration
- accompanying substances

•Delphinidin degrades rapidly in cell culture medium (> 80% loss in 2 h; 37ºC)

•Possible degradation products are: gallic acid and phloroglucinaldehyde

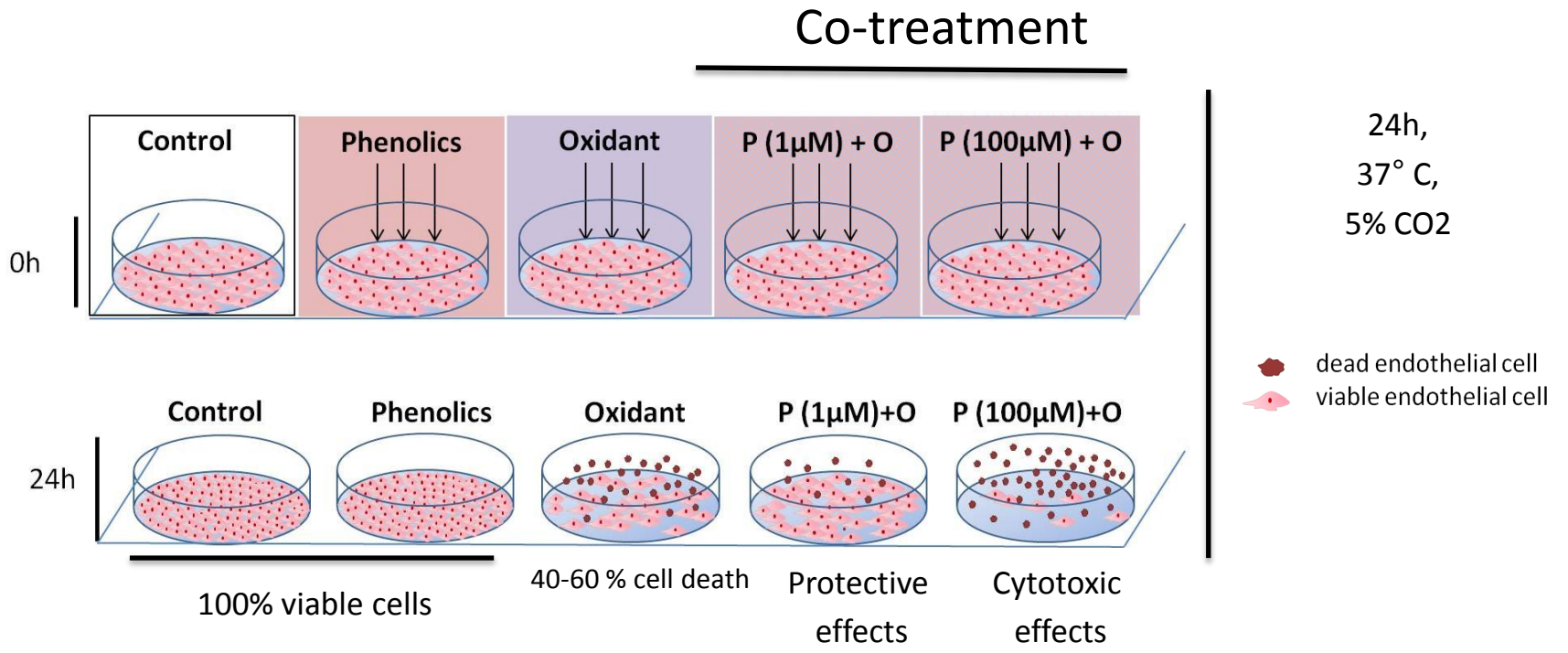
Media	pH	Delphinidin max absorbance [nm]
Cell culture medium (phenol red -free)	7.37	585
Trizma HCl	8.03	605
Acetic acid buffered with sodium acetate	4.23	560

Testing protective effect of selected polyphenols against model of oxidative stress

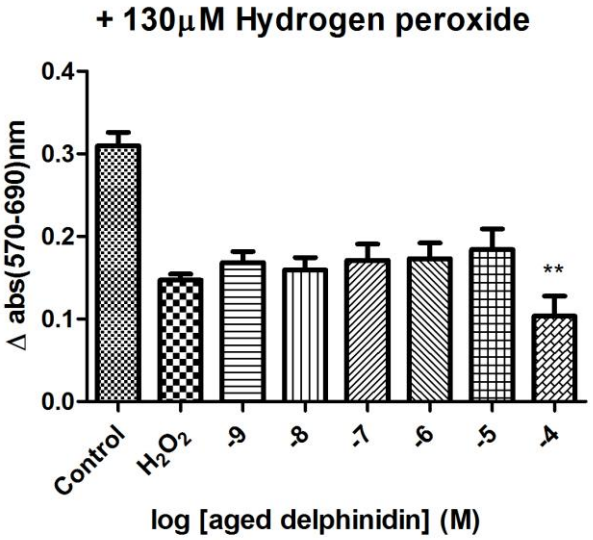
HUVECs (human umbilical endothelial cells)
80-90% confluent cells used for treatment
Oxidants caussed approx. 40-60% cell death

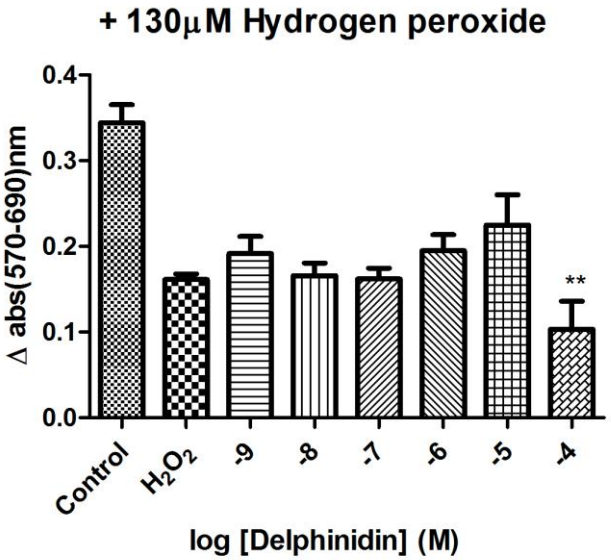
Oxidants	Concentrations
H_2O_2	130µM
Pyrogallol ($OH^{\cdot -}$)	140µM

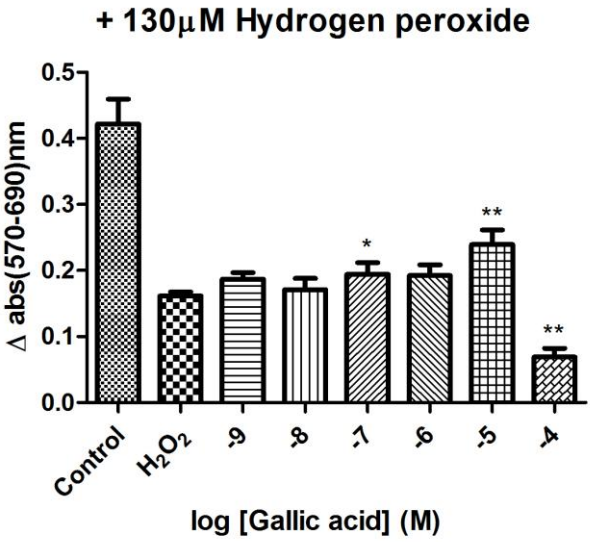
Phenolics	Concentrations
Delphinidin	1nM, 10nM, 100nM, 1µM, 10µM, 100µM,
"Aged delphinidin"	
Gallic acid	
THBA	



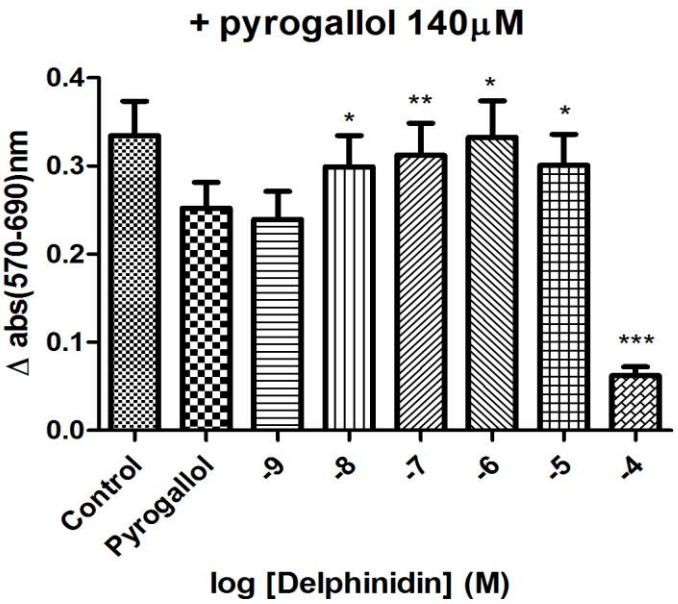
Phenolics vs Hydrogen Peroxide

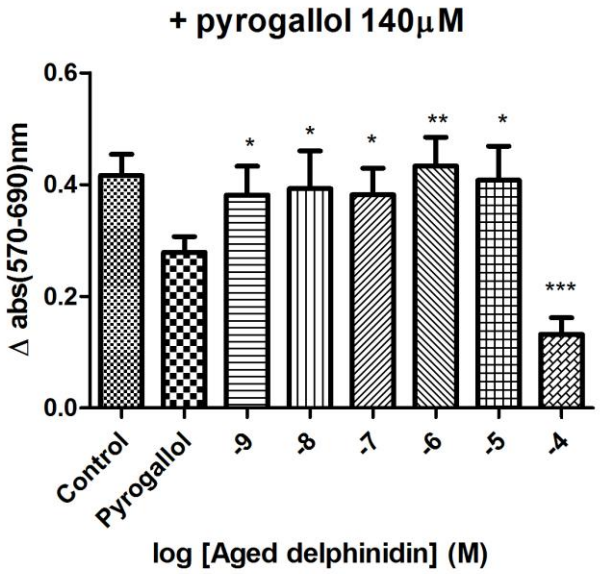


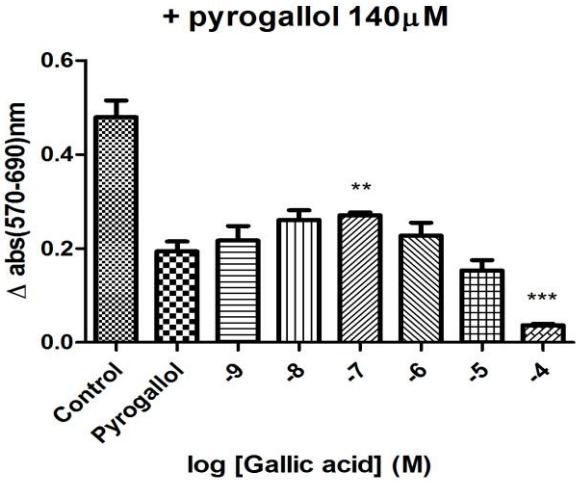




Phenolics vs Pyrogallol ($OH^{\cdot -}$)







Faecal metabolism of berry polyphenols

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But not the same subjects

Phenylacetic acid increased in 7/10 subjects

4-Hydroxy phenylacetic acid increased in 6/10 subjects

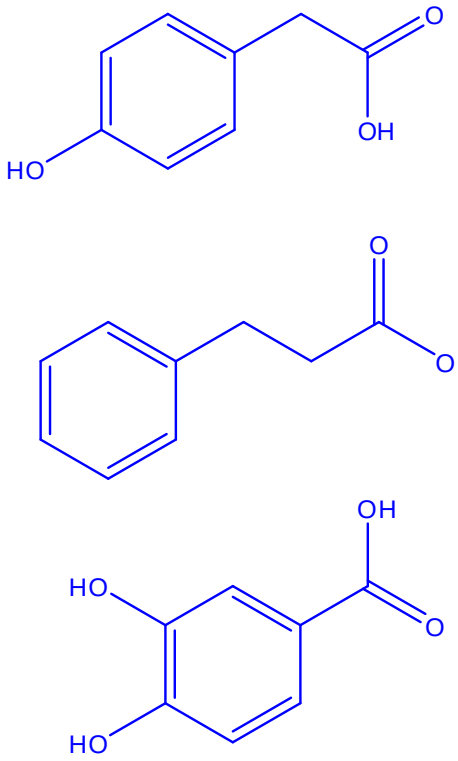
3-Hydroxy phenylacetic acid increased in 5/10 subjects

3-Phenylpropionic acid increased in 6/10 subjects

3(4-Hydroxy)-phenylpropionic acid increased in 5/10 subjects

3,4-Dihydroxy benzoic acid increased in 7/10 subjects

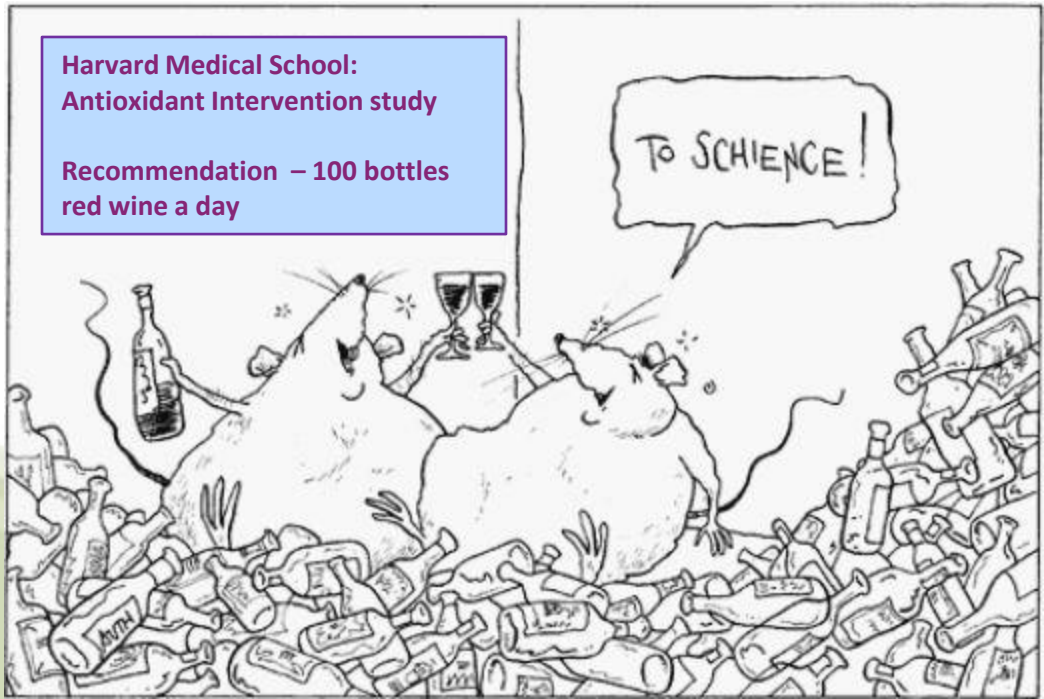
4-Hydroxy benzoic acid increased in 2/10 subjects



- Fits evidence from model studies with faecal inocula but shows large inter-individual variation
- Due to differences in diet or microflora?

Conclusions

- Caution with labelling and claiming that polyphenols have antioxidant-driven health value
- Need of conducting *in vitro/vivo* studies with relevant metabolites of polyphenols; Delphinidin Vs Metabolites
- Gallic acid rather the parent compound itself can be responsible for health beneficial effects
- And at appropriate concentrations in order to fully ascertain their mode of action *in vivo*
- Polyphenols have significant cytotoxic effects at concentrations at or above 10 µM
- Need a corroborating analytical system to establish the definitive derivation of blackcurrant. Stable isotope analysis of blackcurrant AND biological fluids



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Dr Gary Dobson (JHI)

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Alex Foito

Sean Conner

Katarzyna Goszcz