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ANTICARCINOGENIC EFFECT OF PHYTOSTEROLS AND/OR BETA-CRYPTOXANTHIN IN COLON CANCER CELLS

Antonio Cilla Tatay PhD
Postdoctoral researcher
E-mail: antonio.cilla@uv.es

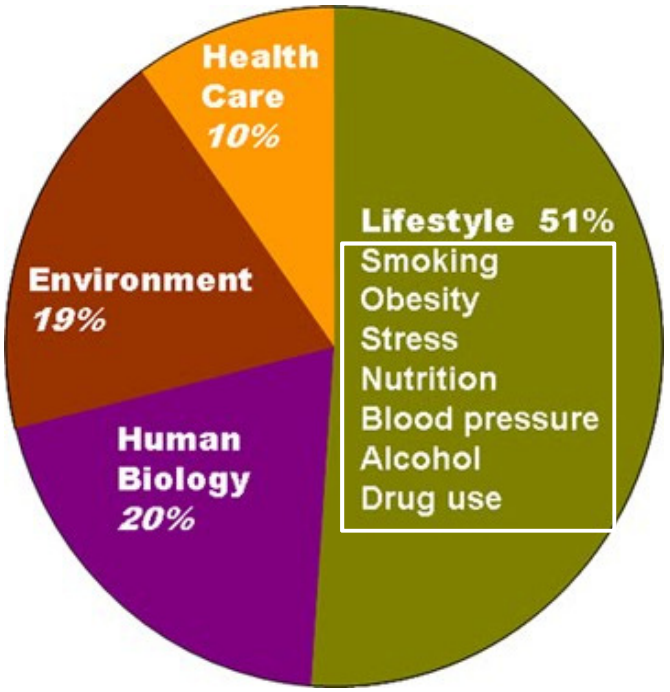
Nutrition and Food Science Area.
Faculty of Pharmacy. University of Valencia

BACKGROUND

"Let food be thy medicine and medicine be thy food"

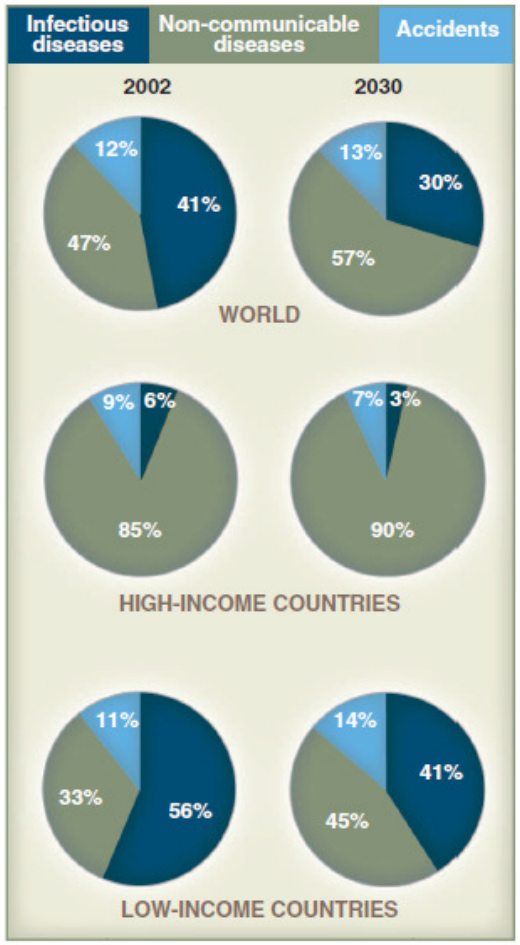
Hippocrates

DETERMINANTS OF HEALTH

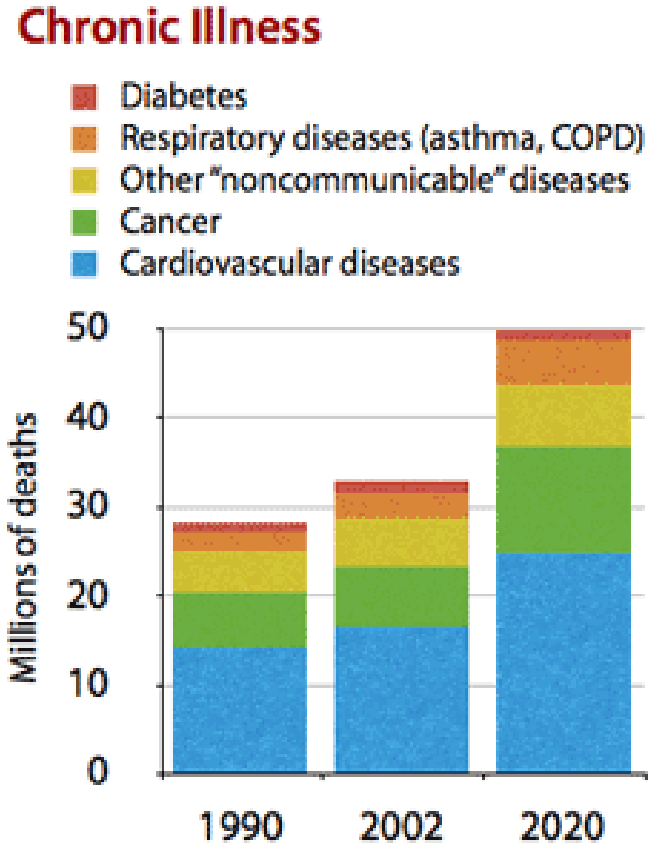


Lalonde (1974)

BACKGROUND



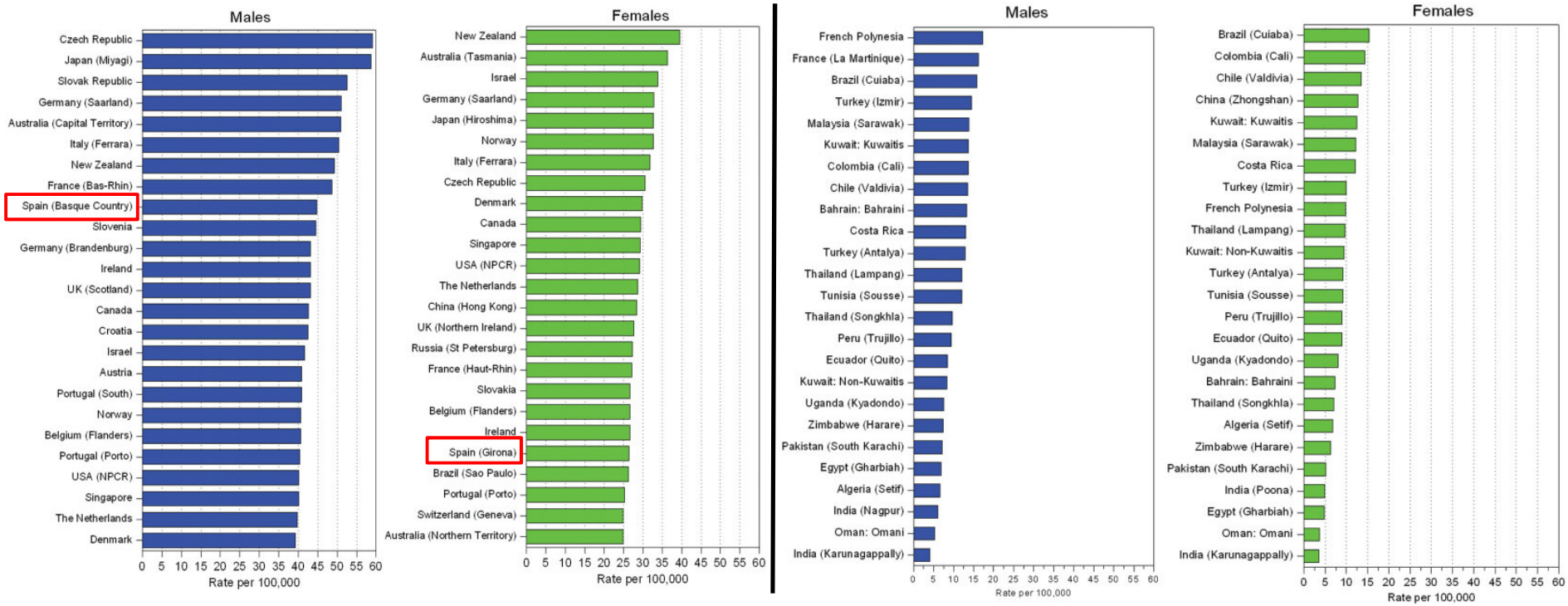
Bygbjerg, I.C. *Science* (2012) 337: 1499-1501



Yach et al. *JAMA* (2004) 291: 2616-2622

BACKGROUND

Colorectal cancer incidence rates by sex



Developed countries

Developing countries

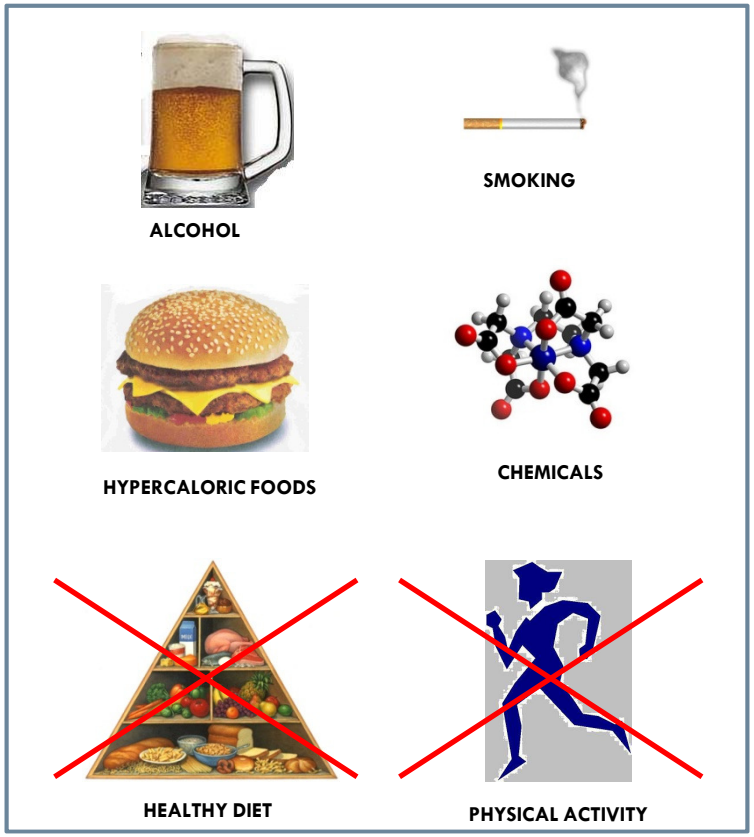
Center et al. *CA Cancer J Clin* (2009)59:366–378

BACKGROUND

Aetiology of colon cancer

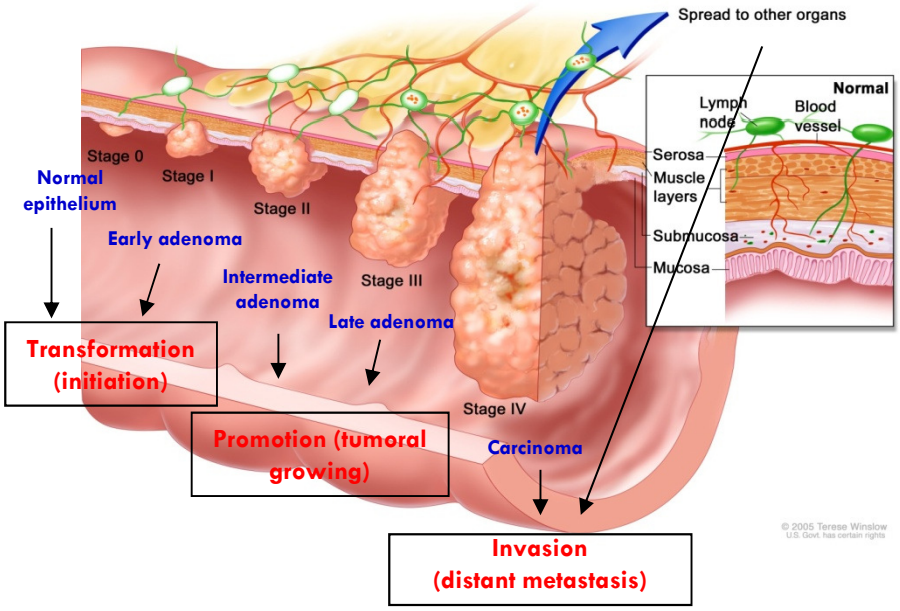
- **Heredity**
- Colorectal cancer is widely believed to be an **enviromental disease**

- Culture
- Social status
- Lifestyle practices → **Dietary habits**

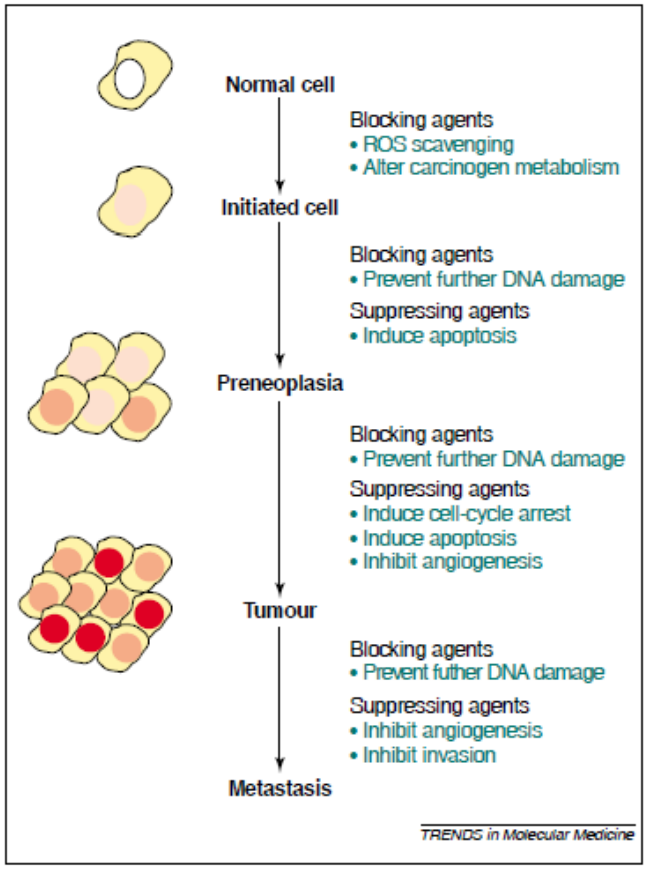


BACKGROUND

Steps and evolution of colon cancer



Chemoprevention by phytochemicals



Manson, M.M. *Trends Mol. Med.* (2003) 9: 11-18

BACKGROUND

Functional food trends in 2014

http://www.foodmag.com.au/news/naturally-functional-foods-to-lead-the-way-in-2014

BACKGROUND

Functional foods

Classical

- Vitamins
- Minerals
- Aminoacids
- Probiotics

Fiber & carbohydrates

- Cereals
- β-glucans
- Soy
- Resistant starch
- Inulin
- Fruit fiber

Bioactive

- Phytosterols
- Carotenoids
- Polyphenols
- Antioxidant extracts (cocoa, grape seed)
- ω-3 and ω-6

β-sitosterol , campesterol and stigmasterol

β-cryptoxanthin

Saura-Calixto et al., (2005)

BACKGROUND

WHY THESE COMPOUNDS?

Gastrointestinal and systemic effects of a doubly modified functional beverage containing β-cryptoxanthin and phytosterols; unravelling potential mechanisms and mediators.

National Research Project (AGL2012-39503-C02-01)

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

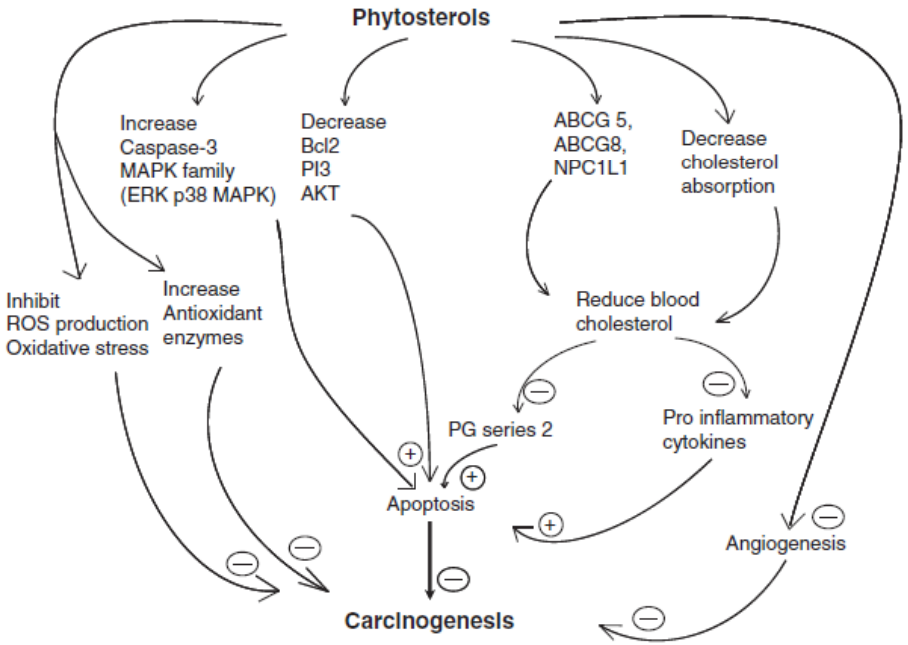
β-cryptoxanthin ⟹ beneficial properties in different bone remodeling markers (formation and resorption)

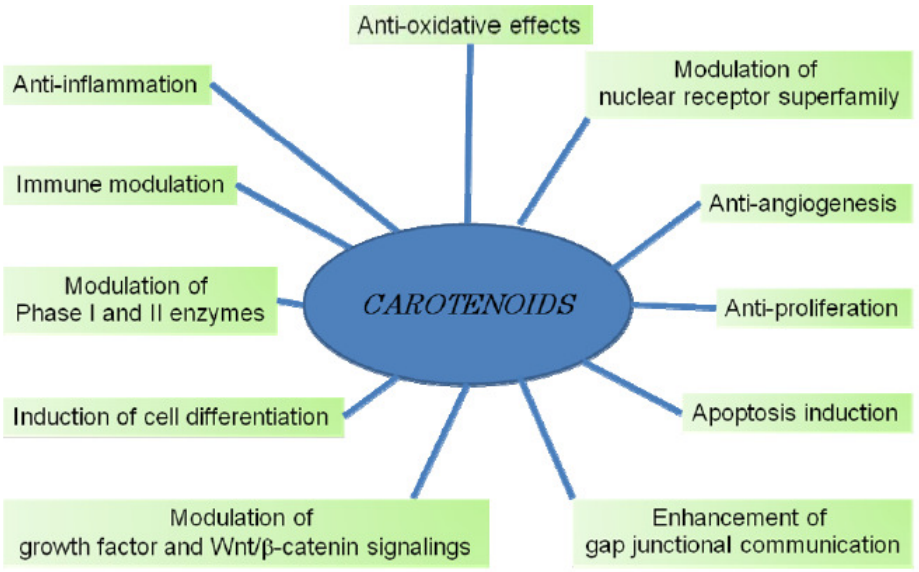
Phytosterols ⟹ hypocholesterolemic effect

Combined action against colon cancer?

BACKGROUND

Proposed mechanisms of action of phytosterols and carotenoids on carcinogenesis





Woyengo, et al. ***Eur. J. Clin. Nutr.*** **(2009) 63: 813-820**

Tanaka et al. ***Molecules*** **(2012) 17: 3202-3242**

OBJECTIVES

1. Evaluate the **antiproliferative activity** of main dietary phytosterols and/or β-cryptoxanthin against a colon cancer cell line (Caco-2 cells).

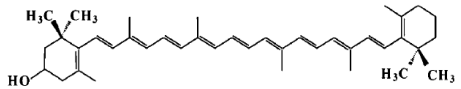
2. Unravel the **biochemical and molecular mechanisms** involved in their possible antiproliferative activity.

SAMPLES AND METHODS

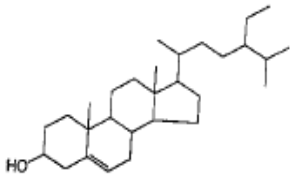
SAMPLES

Standards (alone or in combination) at **phyisiological human serum concentrations** according to:

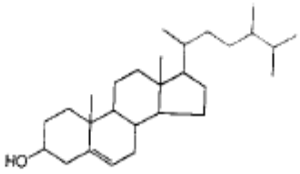
- **Cilla et al., *Ann Nutr. Metab.* (2011) 58, suppl 3, 104**
- **Granado-Lorencio et al., *J. Agric. Food Chem.* (2011) 59, 11819-24**



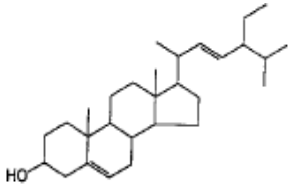
β-cryptoxanthin



β-sitosterol



Campesterol

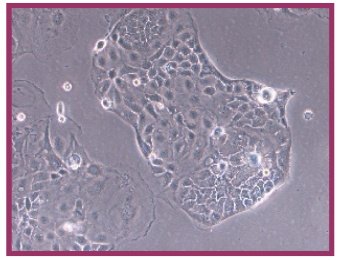


Stigmasterol

METHODS

Cell treatment

(continuous incubation 24 h with phytochemical standards)

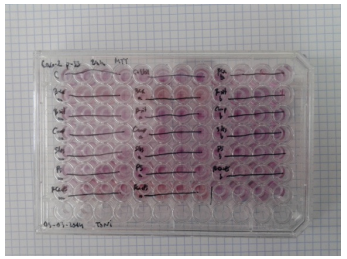


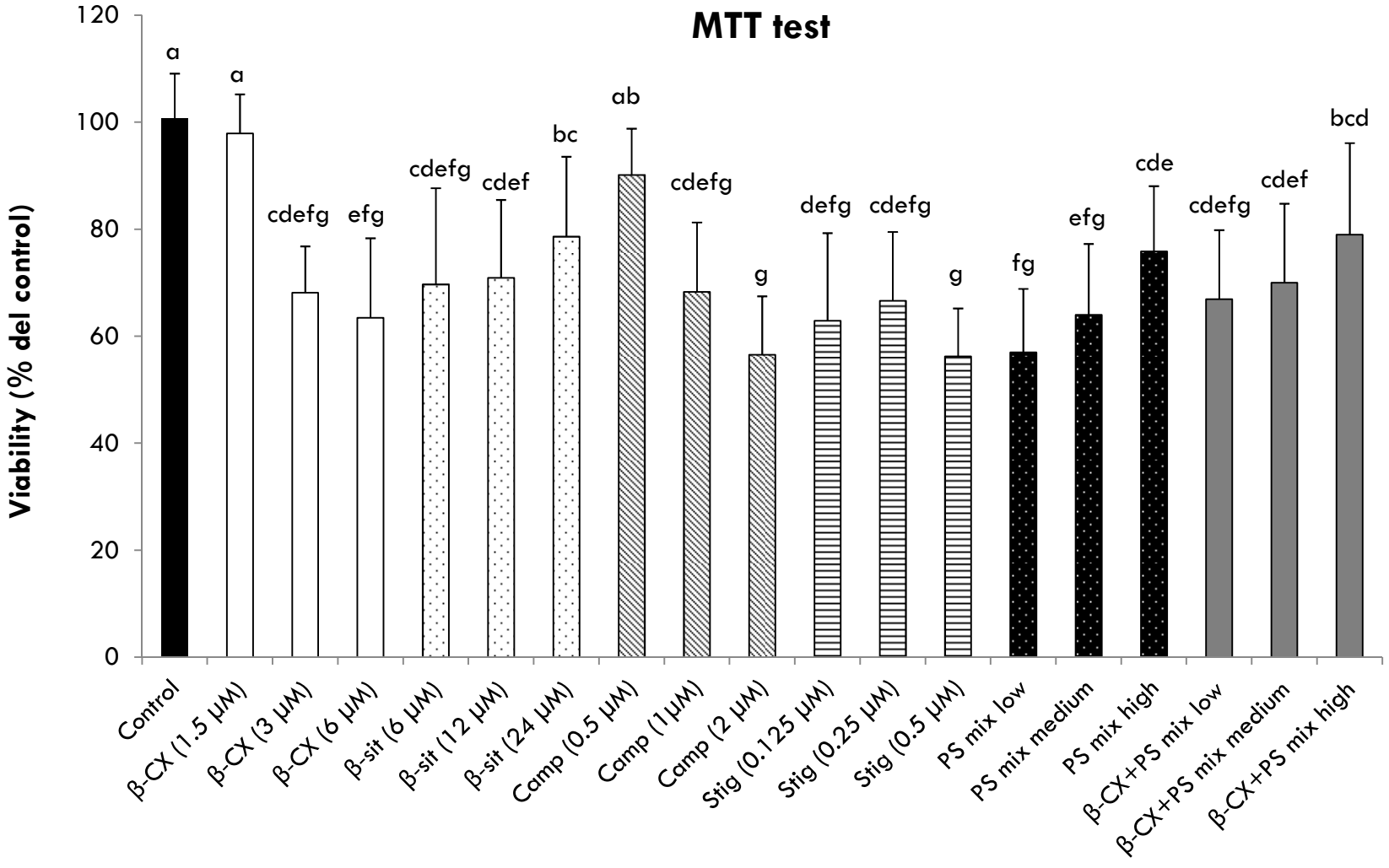
Caco-2 cells

- Cell viability - MTT test (Spectrophotometry)
- Cell cycle (Flow cytometry)
- Apoptosis Annexin V (Flow cytometry)
- Mitochondrial membrane potential (Flow cytometry)
- Intracellular Ca^{2+} (Flow cytometry)
- Reactive oxygen species ROS (Flow cytometry)
- Total thiols (Spectrophotometry)
- Pro-apoptotic BAD protein (Western blot)
- PARP-1 / caspase-3 susbtrate (Western blot)

RESULTS

A) IMPACT ON VIABILITY AND APOPTOSIS





Different letters denote statistical significant differences ($p<0.05$) using one-way ANOVA followed by LSD *post-hoc* test.

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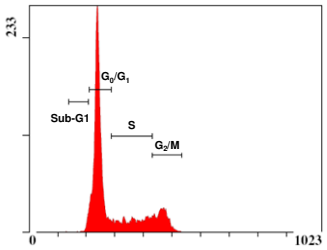
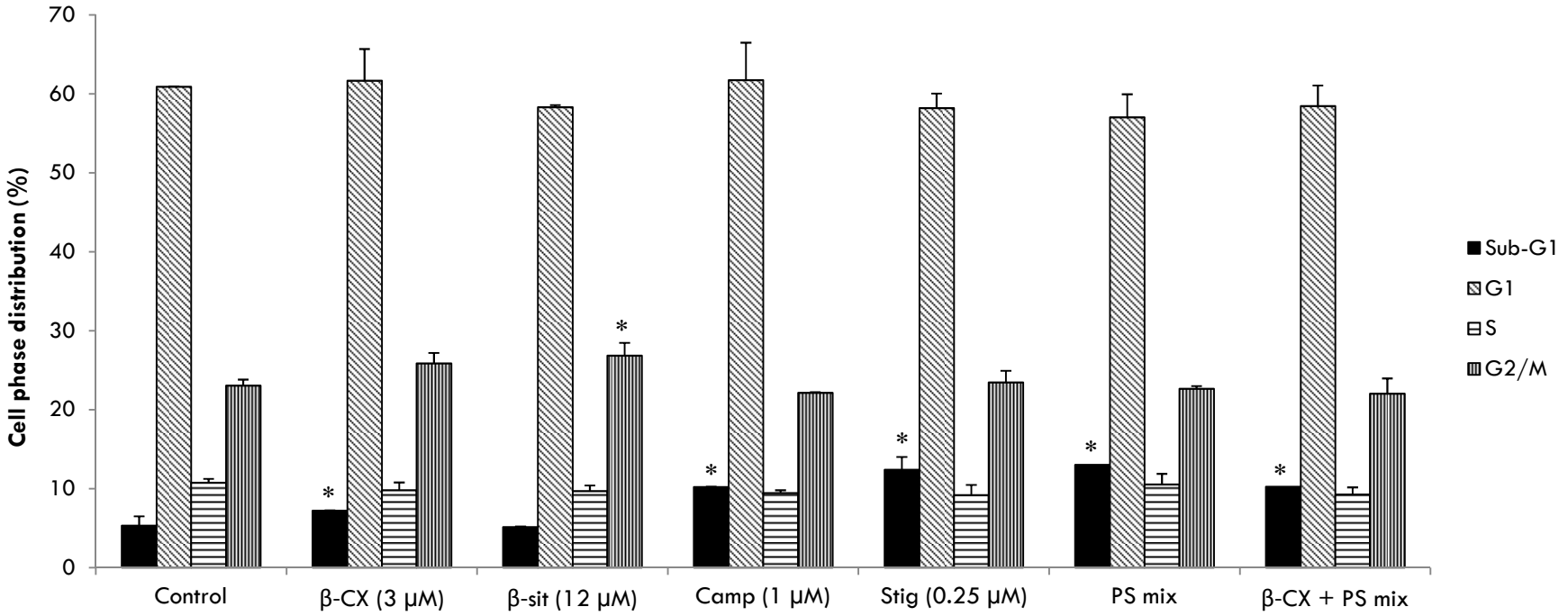


Cell cycle distribution



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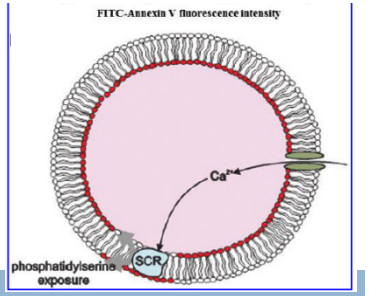
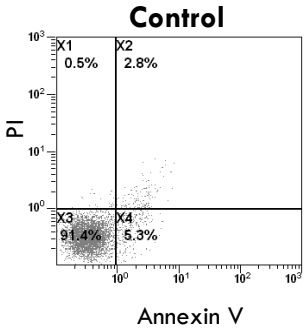
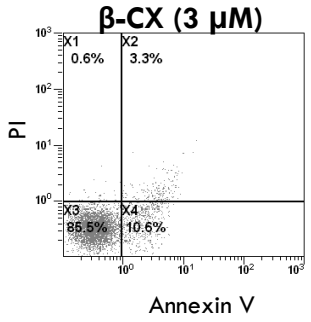
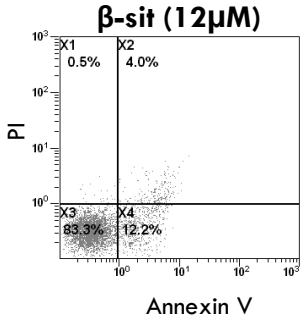
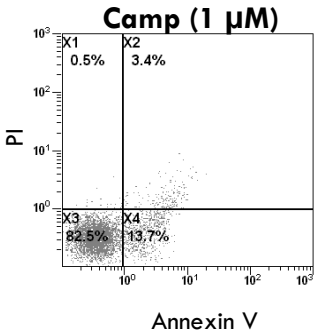


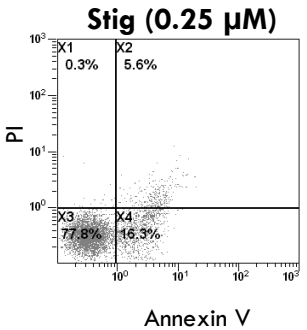
Apoptosis (Annexin V)

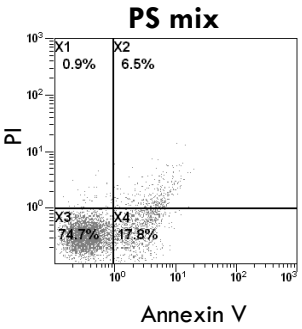


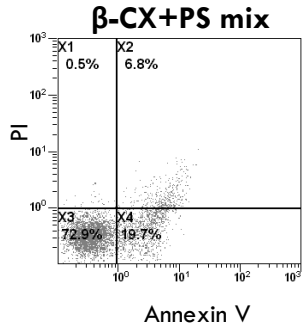












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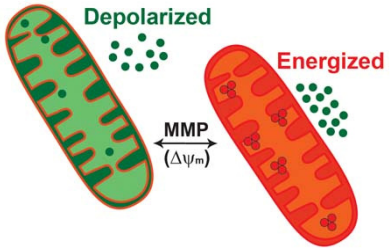
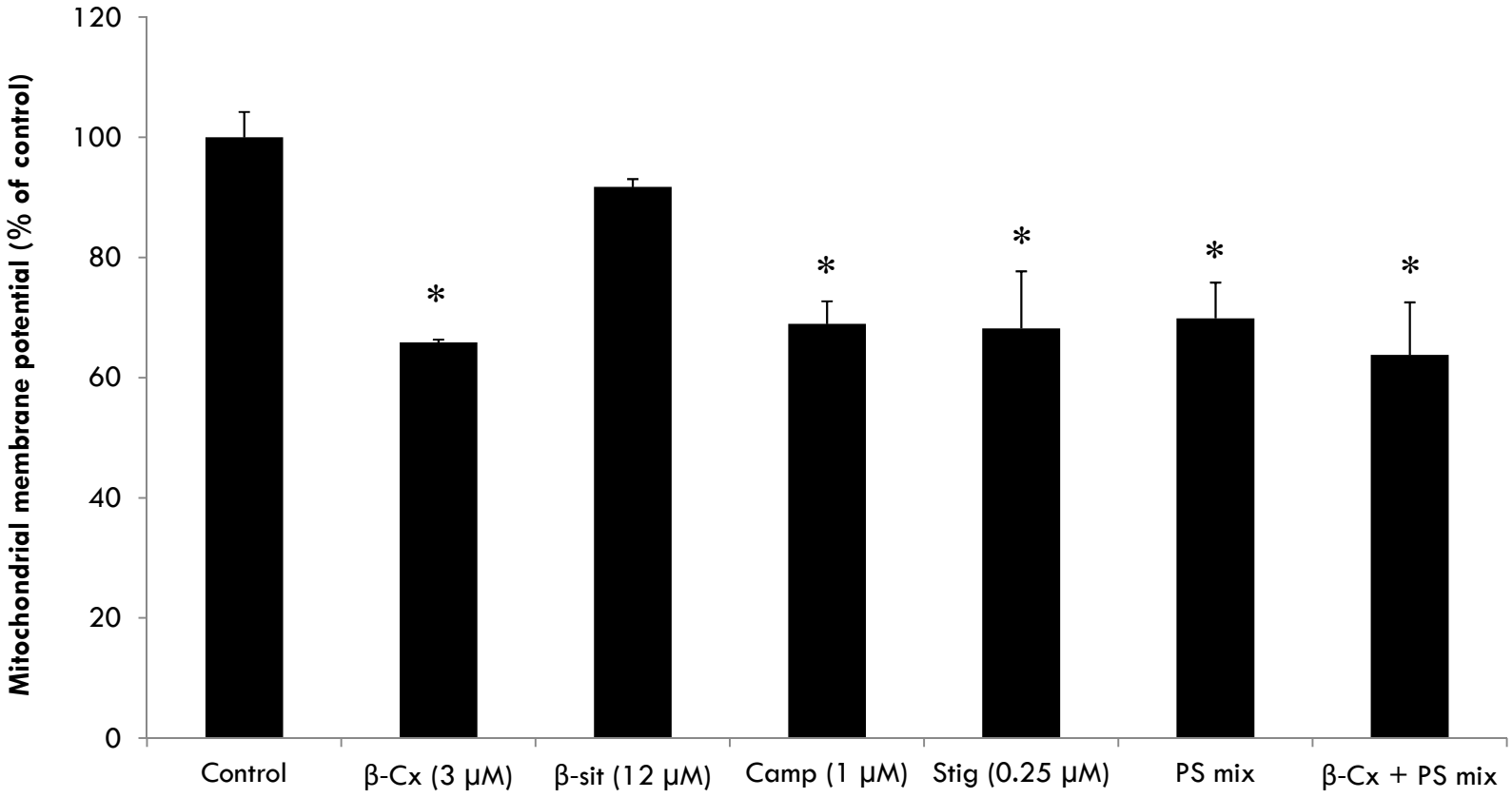


Changes in mitochondrial membrane potential (MMP)



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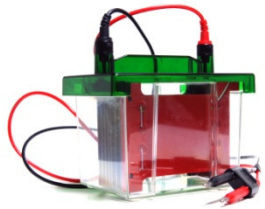
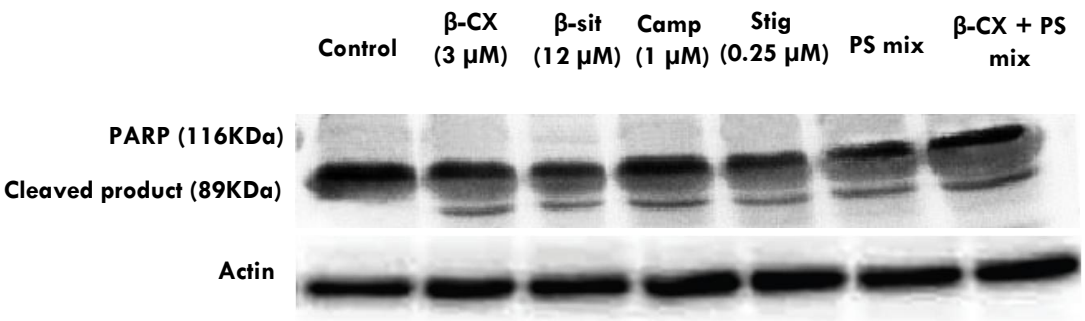
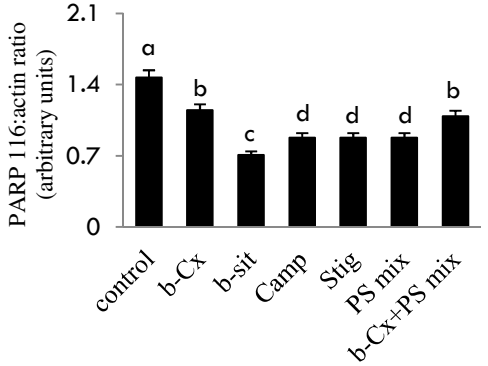


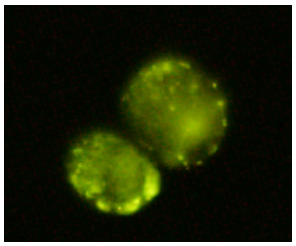
Poly(ADP-ribose)polymerase (PARP) cleavage



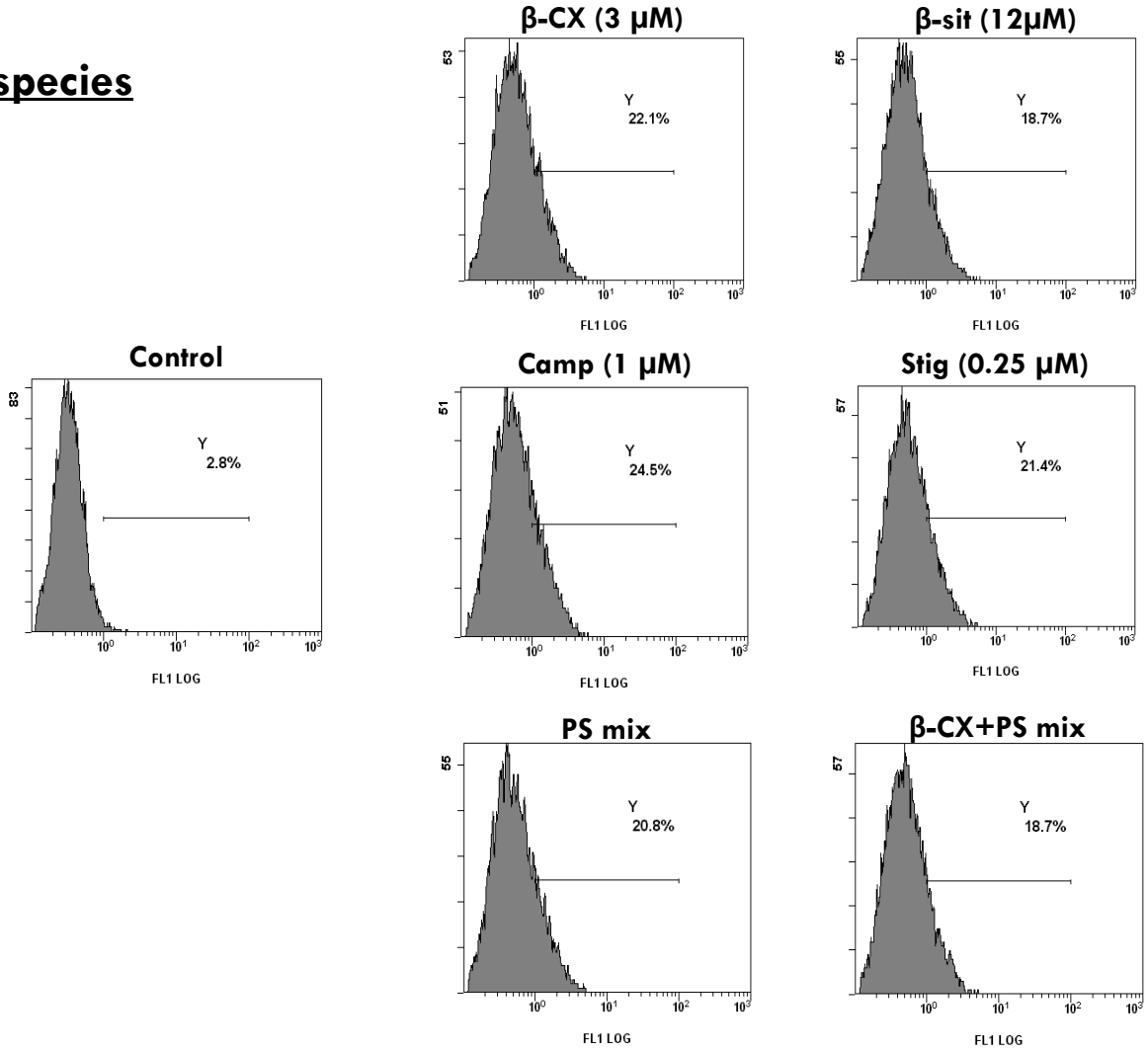


RESULTS

B) REDOX STATE



Reactive oxygen species (ROS)



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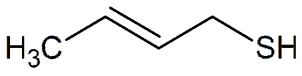

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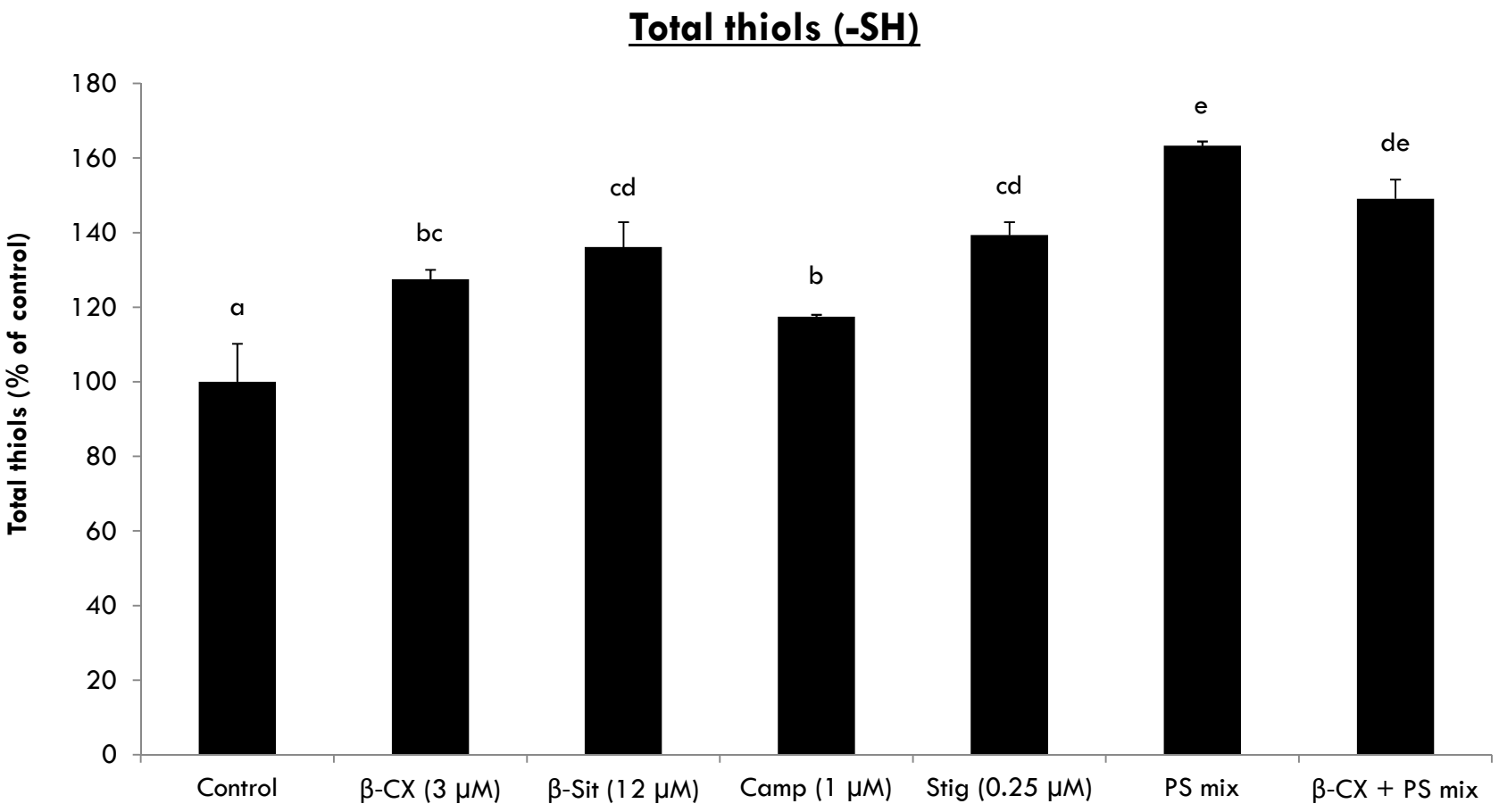
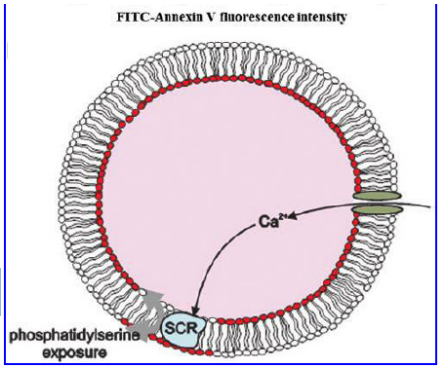
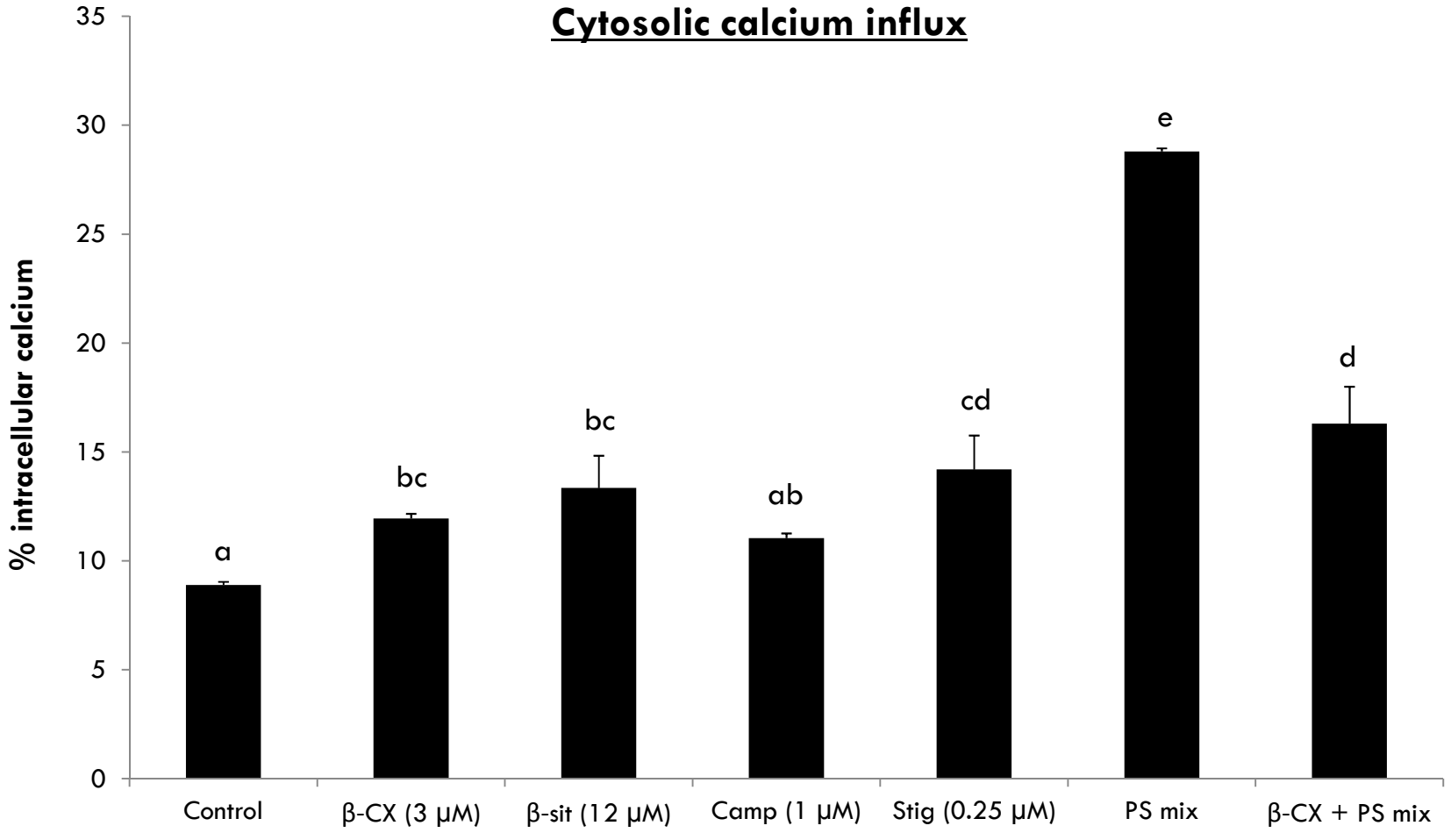


Different letters (a-e) denote statistical significant differences ($p<0.05$) using one-way ANOVA followed by LSD *post-hoc* test.

RESULTS

C) Ca^{2+} AND RELATED SIGNALLING PATHWAYS





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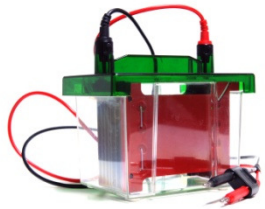
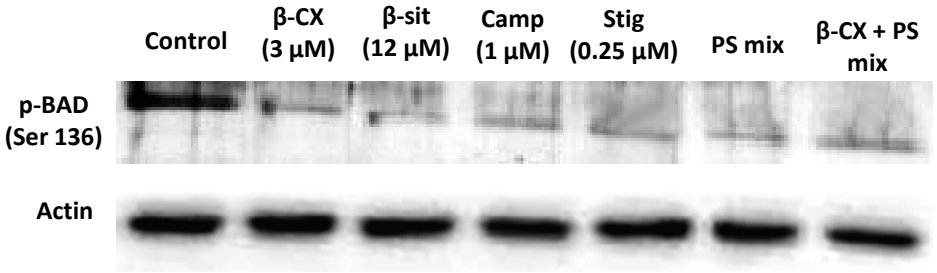
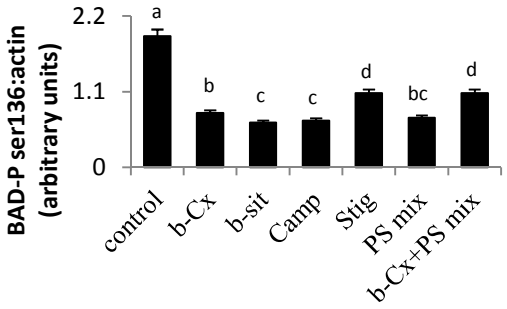


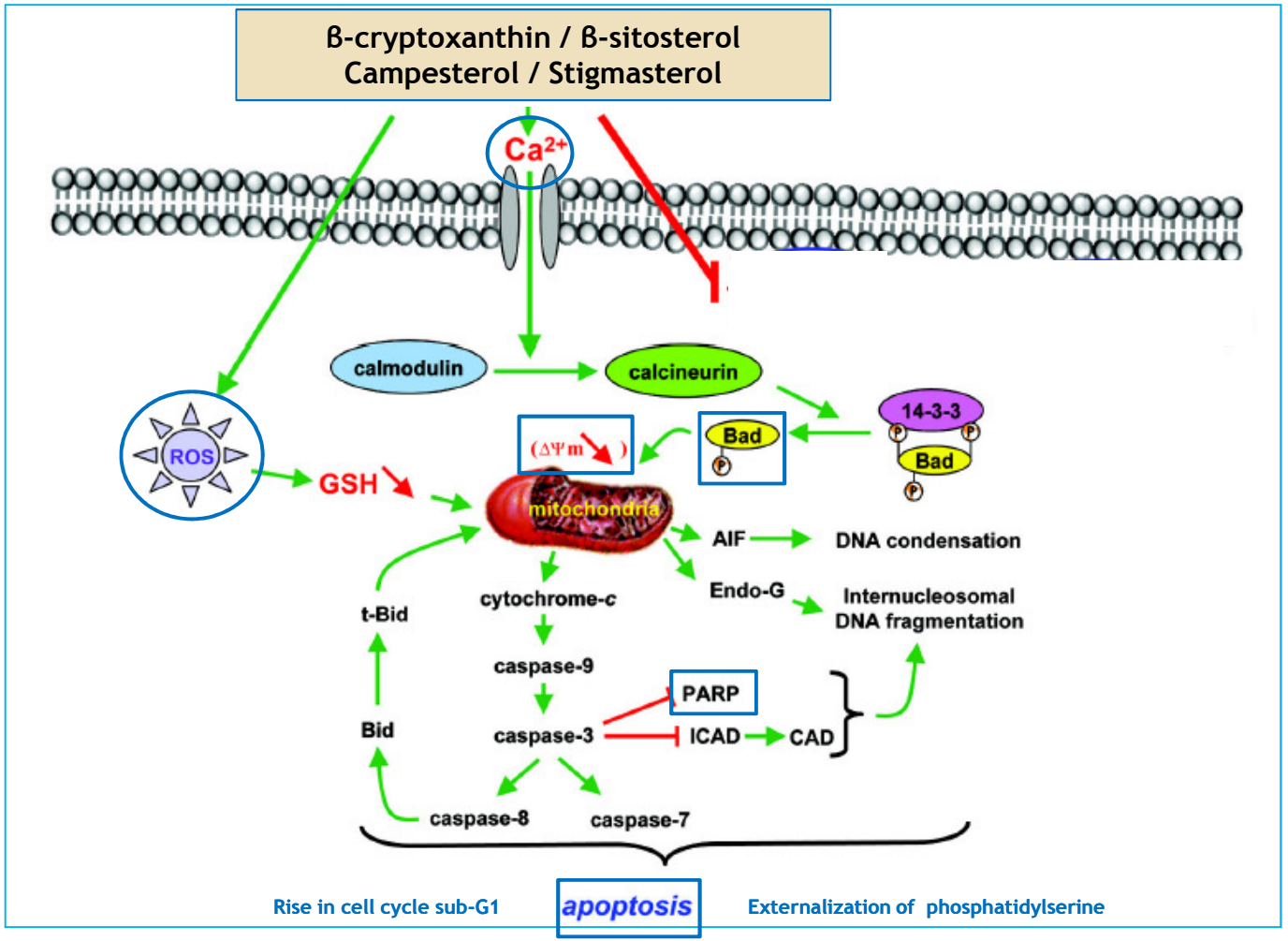
Pro-apoptotic BAD dephosphorilation





RESULTS

OVERVIEW



Adapted from Vejux et al., *Braz. J. Med. Biol. Res.* (2008) 41, 545-556

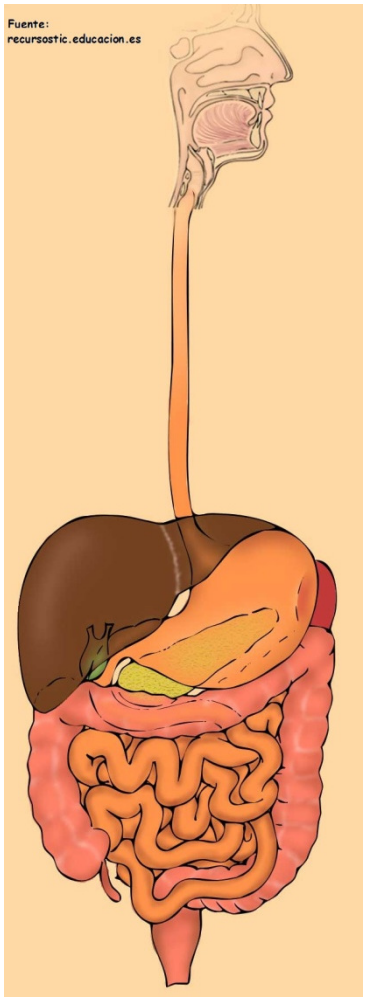
CONCLUSIONS

- β-cryptoxanthin and/or main dietary phytosterols (alone and in combination) reduced cell growth of colon cancer Caco-2 cells up to 44% due to apoptosis, possibly through the mitochondrial pathway

- No clear dose-response was observed, neither additive nor synergistic effect for mixtures, but they retain the same antiproliferative activity as individual compounds indicating absence of antagonistic actions

- The effects were obtained with concentrations compatible with physiological serum levels in humans and with reported bioavailability of these phytochemicals after regular consumption of a beverage containing a mix of all these molecules

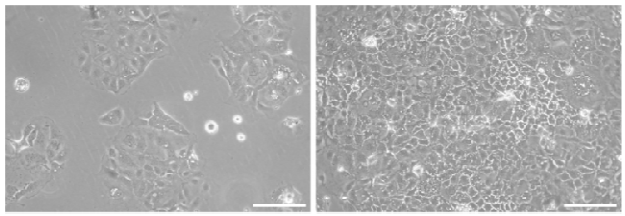
FUTURE RESEARCH



Functional beverages containing phytosterols and β-cryptoxanthin

- In vitro gastrointestinal digestion
- In vitro colonic fermentation

Anti-proliferative activity



Colon cancer (Caco-2 cells)

ACKNOWLEDGEMENTS

Bionutest research group.
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- Dr. María Jesús Lagarda
- Dr. Amparo Alegría
- Dr. Guadalupe García- Llatas
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Laboratory of Biochemistry (STEBICEF).
University of Palermo

- **Dr. Maria Antonia Livrea**
- **Dr. Luisa Tesoriere**
- **Dr. Alessandro Attanzio**
- Dr. Mario Allegra

Thank you for your attention