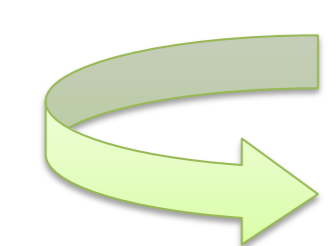


AIM

Investigate the preventive anti-inflammatory activity of two polyphenolic-rich extracts (PREs) derived from *Opuntia ficus-indica* (cactus pear) and *Prunus avium* (sweet cherry) in an *in vitro* cell-based model of intestinal inflammation

Introduction

- Inflammatory Bowel Diseases (IBD) are chronic intestinal disorders with high incidence and prevalence in developed countries¹.
- Pathogenesis of IBD generally includes: Intestinal barrier dysfunction, excessive release of pro-inflammatory mediators and hyperactivation of NF-κB pathway¹.
- Oxidative stress plays a major role in amplification and perpetuation of inflammatory cascades².
- Conventional therapy involves multiple medications and life-long treatments that are associated with severe side effects and high costs³.
- Urgent need to find new and safe compounds to prevent or treat IBD³.



Polyphenols as natural anti-inflammatory alternatives in IBD³!



Cactus pears and cherries are rich sources of phenolic compounds reported as anti-inflammatory and antioxidants^{4,5}.

PREs Characterization

Preparation of cactus pear's and cherry's PRE



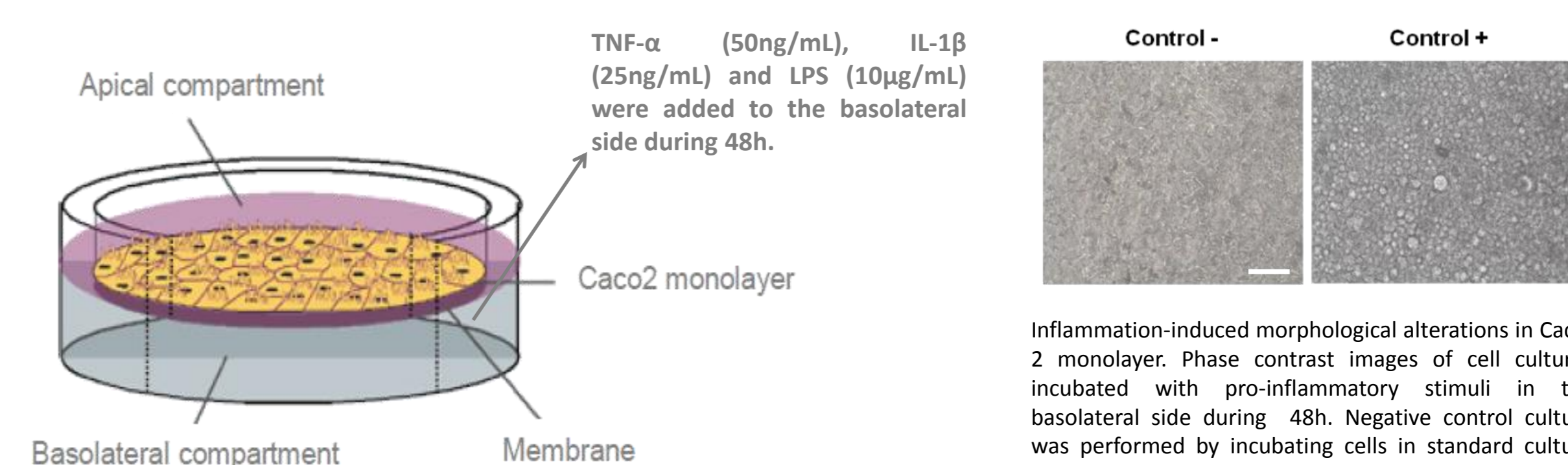
Polyphenolic composition

	Cherry PRE	Cactus pear PRE
Total polyphenols (mg GAE/g extract)	456.9 ± 13.8	171.2 ± 3.3
Total flavonoids (mg CE/mg polyphenols)	0.34 ± 0.03	0.05 ± 0.00

➤ **Cherry PRE** – Mainly composed by **Anthocyanins** that are compounds recognized for their anti-inflammatory capacity⁵.

➤ **Cactus pear PRE** – Mainly composed by **Isorhamnetins and derivatives** that are compounds with anti-inflammatory activity beyond their antioxidant capacity. Also possess in its composition a Betalain, namely Indicaxanthin, known for their anti-inflammatory activity⁴.

IBD Cell-model



Fully differentiated Caco-2 cells stimulated by 50ng/mL **TNF-α**, 25ng/mL **IL-1β** and 10μg/mL **LPS** applied at the basolateral side during 48h can mimic IBD main features⁶:

- Induction of barrier dysfunction,
- Increase in IL-8 secretion,
- Low induction of IL-10 secretion,
- Increase in NO secretion,
- NF-κB pathway activation.

Preventive Anti-inflammatory Activity

Fully differentiated Caco-2 cells were pre-incubated with cherry's or cactus pear's PRE (50mg GAE/L) during 4 hours followed by stimulation with pro-inflammatory cocktail (50ng/mL TNF-α, 25ng/mL IL-1β and 10μg/mL)⁶.

Barrier Dysfunction

Barrier dysfunction was measured by alterations in fluorescein permeability across Caco-2 monolayer.

	P _{app} 48 hours (cm/s)	
	Apical → Basolateral	Basolateral → Apical
Control - *	6,04x10 ⁻⁶	4,58x10 ⁻⁶
Control + **	7,39x10 ⁻⁶	8,47x10 ⁻⁶
Cactus pear PRE	5,58x10 ⁻⁶	6,41x10 ⁻⁶
Cherry PRE	6,38x10 ⁻⁶	5,40x10 ⁻⁶

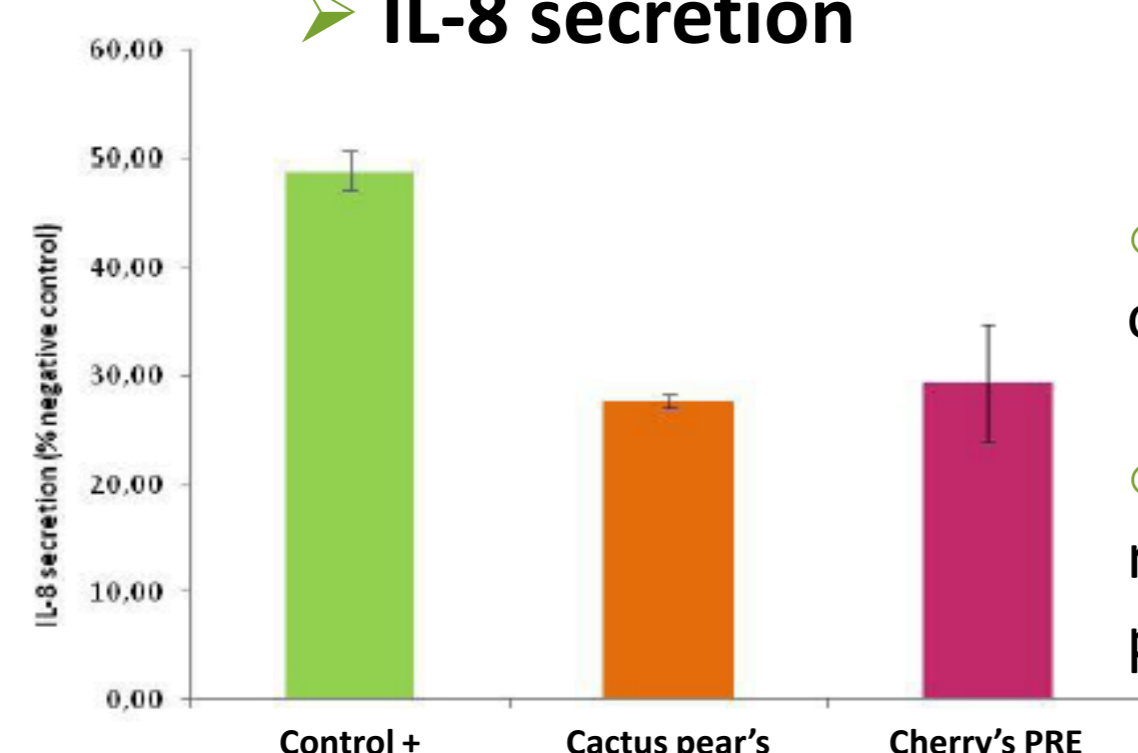
* Negative control represents cells without incubation with pro-inflammatory stimuli.
** Positive control represents cells with incubation with pro-inflammatory stimuli.

Permeability of fluorescein across inflamed Caco-2 monolayer after pre-incubation with Cactus pear and Cherry PRE (50mg GAE/L).

Both PRE could protect against barrier dysfunction but in different directions:

- **Cherry PRE** was more effective in basolateral to apical direction.
- **Cactus pear PRE** had higher protection in apical to basolateral direction.

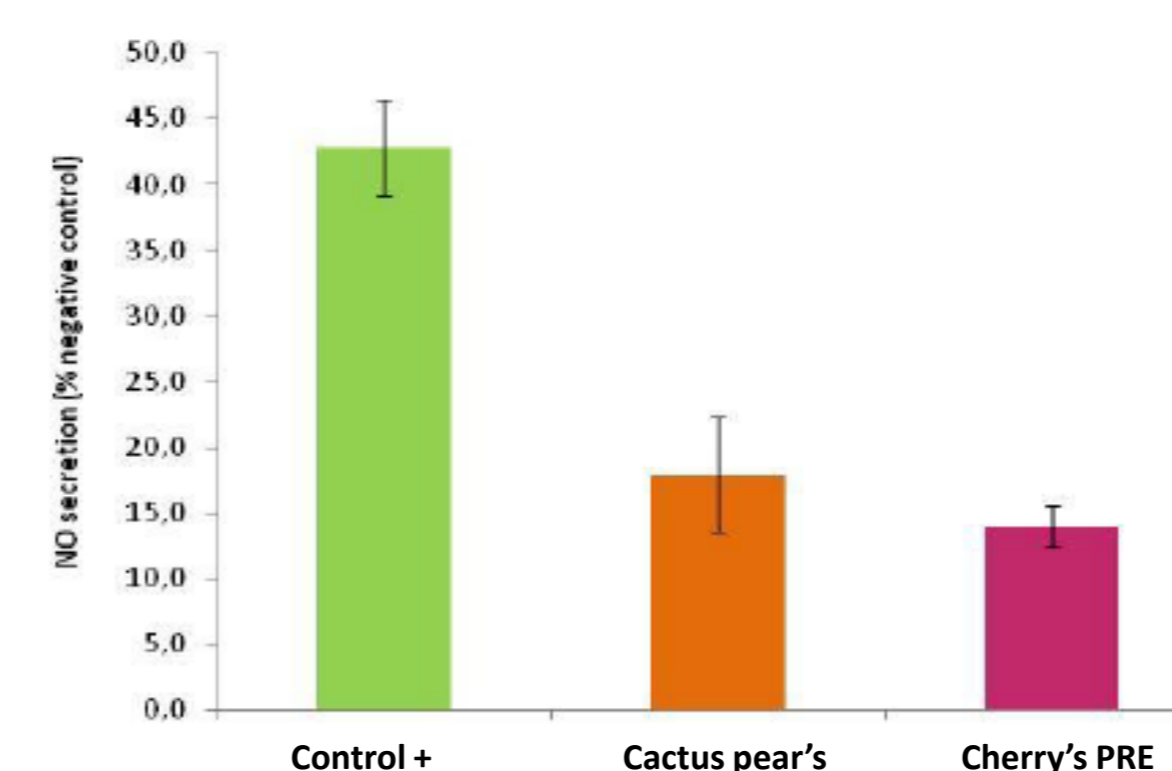
IL-8 secretion



- IL-8 is a chemoattractive cytokine that is overexpressed in IBD¹.
- Cactus pear's and cherry's PRE can reduce IL-8 secretion in a similar percentage.

Both extracts could reduce IL-8 secretion

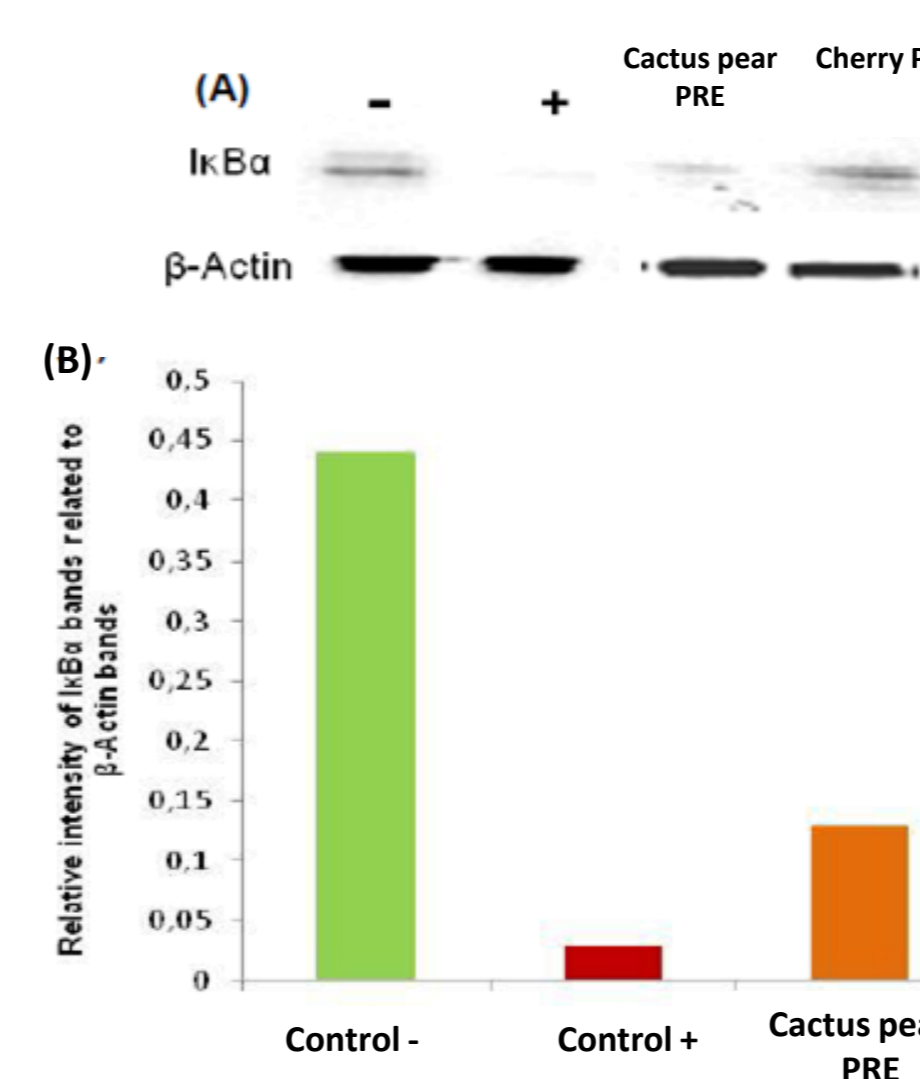
NO secretion



- Nitric oxide (NO) is an important inflammatory mediator in IBD¹.
- Cactus pear's and cherry's PRE were able to reduce NO secretion.

Cactus pear's and cherry's PRE could decrease NO secretion

NF-κB activation



- NF-κB activation was measured indirectly by determination of IκBα levels.
- Cactus pear's and cherry's PRE could modulate NF-κB activation but **cherry's PRE** was more effective.

Both PRE could modulate NF-κB activation

Conclusion

- Cactus pear's and Cherry's polyphenolic-rich extracts could modulate inflammatory mediators in an *in vitro* cell-based model of intestinal inflammation.
- Since oxidative stress is an activator of NF-κB pathway, this is one possible mechanism of action of both extracts⁷.
- Possibly, PREs could control IL-8 and NO secretion through a mechanism depended on NF-κB.
- **Both extracts are promising agents in prevention of IBD.**

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