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# ANTIDIARRHOEAL AGENTS AND PARACELLULAR PERMEABILITY OF E. COLI INFECTED CACO-GOBLET INTESTINAL MODEL

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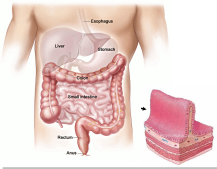
## BACKGROUND

Diarrhoea is an important cause of morbidity and mortality worldwide, in spite of the advances in health technology, improved management, and increased use of oral rehydration solutions in recent decades, persisting as a major cause of death in children under five years of age. The Caco-2 monolayer represents a simplified but robust mucosa with a very efficient barrier; the modified model (Caco-Goblet) including mucous secerning goblet cells is relevant to study transport and mucous interaction taking into account the biological properties of the compounds and to establish predictive experimental model for a preclinical in vitro assessment. It has been demonstrated that gelatine tannate (Tasectan) protects intestinal cells from damage induced by *Escherichia coli* whilst also preventing *E coli* adhesion in Caco-Goblet® intestinal epithelium model. The present study aims to evaluate this dual-inhibitory effect upon *E coli* for a new product combining gelatine tannate with a mixture of probiotics (TSC Duo) as compared to other antidiarrhoeal agents already available in the market.

### *In vivo*



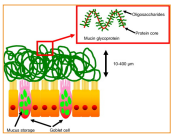
Diarrhoea is located at the small intestine

Characterised by absorption and/or secretions disturbence

Mainly related with Inflammatory reaction caused by bacterial infections

## ESCHERICHIA COLI INVASION MODEL ON Caco-Goblet® cells

### EXPERIMENTAL DESIGN



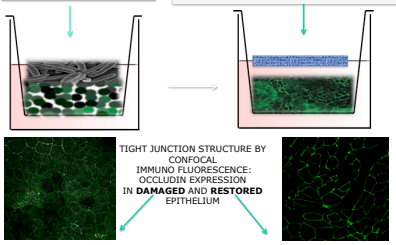
**CacoGoblet™**
ready-to-use model for *in vitro* intestinal absorption evaluation. A 21-days cell barrier is formed by differentiated co-cultured Caco-2 and human goblet mucus secreting cells plated on HTS Transwell permeable supports.
Co-cultured Caco-2 and human goblet mucus secreting cells leads to a more permeable epithelium compared to caco-2 monolayer, better mimicking the in vivo small intestine

***Escherichia coli***
*1x10⁸ cfu/mL*
**30 min**
Inducing the full bacterial invasion

5mg/mL Tasectan Duo & Smecta

12,5 mg/mL Probiotic Mixture &S. boulardii

**TREATMENT: 1h/24h**

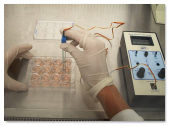


TIGHT JUNCTION STRUCTURE BY CONFOCAL IMMUNO FLUORESCENCE: OCCLUDIN EXPRESSION IN **DAMAGED** AND **RESTORED** EPITHELIUM

CacoGoblets inoculated with *E.coli* (1x10⁸ cfu/mL) have been monitored as positive control serie.
***E.coli*** has been applied for **30 minutes** on CacoGoblet: this time has been previosuly defined as inducing a **reversible TEER reduction.**

Then **products** have been applied for **1h** and **24h** at the doses of:
**·5 mg/mL: Tasectan Duo** and **Smecta**
**·12,5 mg/mL: Probiotic Mixture** and ***Saccaromyces boulardii***
considering the standard dosage according to producer's instructions

## END POINTS: BARRIER PROPERTIES and PERMEABILITY



**PROCEDURE**

Trans epithelial electrical resistance (TEER)

**·Before and after E.coli inoculum & after products treatment**

**·Measure Ion Permeability ➔fence properties**

**·E.COLI inoculum induces a TEER DECREASE**

**·Product treatment: TEER increase > film forming properties**

**To evaluate product efficacy two physical parameters have been used: measurement of TEER and Lucifer yellow assay that respectively measure the efficiency of tight junctions and the paracellular flux within the intestinal barrier.**

**PROCEDURE**

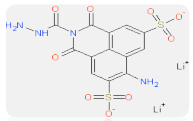
Lucifer Yellow Passage

**·After products treatment**

**·Paracellular Flux Marker➔ Integrity**

**·E.COLI inoculum induces a FLUX INCREASE**

**·Product efficacy: Flux decrease> astringency properties**



## RESULTS

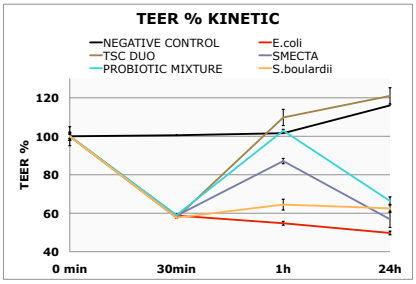
### E. COLI EFFECT ON TIGHT JUNCTIONS

The microorganism's adhesion to the intestinal mucosa is a crucial event for the beginning of the infectious process. One of the consequences of pathogenic bacteria on the brush border is the lesion of the epithelium, which allows the bacteria proliferation and the consequent death of epithelial cells.

·SIGNIFICANT TEER REDUCTION (< 70 OHM*cm2) after 30 minutes from bacteria application

·SIGNIFICANT LUCIFER YELLOW FLUX INCREASE

### TEER % KINETIC




### PRODUCT EFFICACY: RECOVERY BASED ON TEER AND LUCIFER YELLOW

**TEER VALUES & PARACELLULAR FLUX**: the products enhances fence and barrier properties:

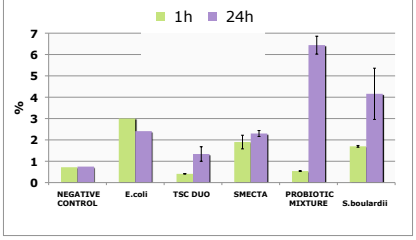
·TEER INCREASE

·LUCIFER YELLOW FLUX DECREASE

**FILM FORMING EFFECT:** the product creates a protective film interacting with Tjs.

**MUCOADHESIVE PROPERTIES**: the compound forms bonds with mucins and is able to adhere to the apical epithelial cells.

### LUCIFER YELLOW PARACELLULAR FLUX




## SUMMARY

| | TASECTAN DUO | | SMECTA | | PROBIOTIC MIXTURE | | S.boulardi | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1h | 24h | 1h | 24h | 1h | 24h | 1h | 24h | |
| FENCE PROPERTIES | ↑ 123% | ↑ 149% | ↑ 69% | ↓ -4,5% | ↑ 107% | ↑ 18% | ↑ 16% | ↑ 11% | RECOVERY TEER% |
| INTEGRITY (%DAMAGE) | 14% ↓ | 56% ↓ | 63% ↓ | 95% ↑ | 18% ↓ | 267% ↑ | 57% ↓ | 172% ↑ | LUCIFER YELLOW FLUX% |

**Negative Control values :** Lucifer Yellow flux%1h =24% Lucifer yellow flux 24h=31%

**Positive Control values :** TEER % 1h=-10% Lucifer Yellow flux%1h=100% TEER % 24h=-22% Lucifer Yellow flux%24h=80%

### PRODUCTS EFFICACY

EFFICACY IN COUNTERACTING E. COLI

- **TASECTAN DUO**
  Strong increase in TEER values and strong decrease in LY flux at both treatment times
  ➔ STRONG protective efficacy in counteracting *E.coli* damage **at 1h and after 24h**
- **PROBIOTIC MIXTURE**
  Strong increase in TEER and strong decrease in LY paracellular flux at 1h
  ➔ STRONG protective efficacy in counteracting *E.coli* damage at 1h
- **SMECTA**
  Slight increase in TEER and slight decrease in LY flux only at 1h
  ➔MODERATE protective efficacy in counteracting *E.coli* at 1h
- ***S. boulardii***
  NOT significant TEER increase and not significant LY flux reduction
  ➔SLIGHT protective efficacy in counteracting *E.coli* at 1h

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