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Combination of probiotics, plant extracts and micronutrients shows positive effects on the integrity of the intestinal barrier

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Functional disorders of the gastrointestinal tract cause symptoms that can have a significant impact on the quality of life. Maintaining an intact intestinal barrier is essential for preventing and treating these conditions. Initial studies show promising results regarding the synergistic effect of a combination of probiotic bacteria and plant extracts.

Dysbiosis causes functional gastrointestinal disorders

The prevalence of functional gastrointestinal disorders (FGIDs) is 40% worldwide [1]. The Rome Foundation defines them as diseases classified by gastrointestinal symptoms associated with an individually variable combination of the following pathophysiologically relevant factors: motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota and altered central nervous system (CNS) signal processing. Rome-IV lists 30 such disorders for adults and 20 for children [2].

Multiple studies have shown that a dysbiosis of the intestinal microbiome is associated with various disorders or could even be the cause of such complaints. The following are examples [1]:

- Influence on intestinal motility
- Increased gas production
- Alteration of the gut-associated immune system
- Irritation/inflammation of the intestinal wall
- Damage to the intestinal barrier function

Disturbances of the cell connections can lead to a permeable intestine (leaky gut). The protective effect of the mucous membrane is then reduced, which can lead to a vicious circle of an increased immune response and further inflammation with renewed damage to the intestinal wall [1].

Combination of probiotics with plant extracts and micronutrients shows versatile bioactivity on FGID pathomechanisms

A combination of living microorganisms (microbiota) and plant extracts was investigated with regard to their bioactivity.

The following study preparations were used:

A) Pegaso® Enterodophilus® Baby (Colikind® drops), Schwabe Pharma, Italy [3, 4]:

Quantities per daily dose	
Lactobacillus reuteri DSM 25175	1×10^9 CFU
Lactobacillus acidophilus DSM 24936	1×10^9 CFU
Chamomilla recutita L. oleolita (drug/extract ratio 1 : 2)	5 mg
Native organic olive oil extra	

B) Pegaso® Enterodophilus® Junior, Schwabe Pharma, Italy [5]:

Quantities per daily dose	
Lactobacillus reuteri DSM 25175	3×10^9 CFU
Lactobacillus acidophilus DSM 24936	3×10^9 CFU
Chamomilla recutita L. dry extract	460 mg
of this, apigenin	5.2 mg
FOS (fructooligosaccharides)	37 mg
Vitamin A	320 µg
Vitamin D	5 µg

As a cell model, a single-layer CaCo-2 cell culture (monolayer of polarised growing colon epithelial cells) was used, which had been pre-treated with interferon gamma (INF-γ) plus tumour necrosis factor alpha (TNF-α) or lipopolysaccharides (LPS) to induce inflammatory processes. These proinflammatory signalling substances contribute in vivo to the development and/or spread of damage to the intestinal barrier, which also manifests itself in an increase in

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Tab. 1. Effect of pre-treatment with the investigated complex on the transepithelial electrical resistance (TEER) after inflammatory stimulation.

Test model	Result	References
CaCo-2 monolayer, pre-treatment with complex A, after 24 hours of inflammatory stimulation with LPS-conditioned THP-1 medium	After 3 and 6 hours, the pre-treatment resulted in a slight relative increase in TEER compared to the inflammatory stimulus of the control, $p < 0.05$	[3]
CaCo-2 monolayer, pre-treatment with complex A, after 24 hours of inflammatory stimulation with LPS	At 21 and 24 hours, pre-treatment increased TEER by 11.43% and 7.78%, respectively, compared to the inflammatory stimulus of the control, $p < 0.001$	[4]
CaCo-2 monolayer, pre-treatment with complex A, after 24 hours of inflammatory stimulation with INF-γ + TNF-α	After 24 hours, the pre-treatment increased the TEER significantly by 9.46% compared to the inflammatory stimulus of the control, $p < 0.05$	[4]
CaCo-2 monolayer, pre-treatment with complex B, after 24 hours of inflammation stimulation with LPS	After 24 hours, pre-treatment increased TEER by 10.99% compared to the inflammatory stimulus of the control (personal information[1]), $p < 0.05$	[5]

CaCo: Colon carcinoma; LPS: Lipopolysaccharide; THP-1: Human monocytic cell line; INF: Interferon; TNF: Tumour necrosis factor

permeability (scientific term: permeability disorder; common English: leaky gut). The CaCo-2 monolayer is an established cell culture model to study the pharmacological modulation of the epithelial barrier and the integrity of tight junctions (cell contacts).

Based on this model, transepithelial electrical resistance (TEER) and paracellular permeability are considered specific and sensitive biomarkers of intestinal barrier integrity and function. Lesions in the cell culture cause a drop in impedance in the TEER test. Treatment with inflammatory stimulants lowered the TEER compared to the untreated control, while pre-treatment with the study drug lowered the percentage resistance to a lesser extent. This resulted in a relative increase in TEER after pre-treatment with the drug complexes compared to the more reduced TEER of the control groups (see **Tab. 1**). These results were confirmed by determining the fluorescein isothyanate flux through the cell layer following the TEER experiment (see **Fig. 1**) [3–5].

In another in vitro model, THP-1 cells were stimulated with LPS and the release of pro-inflammatory cytokines was determined. Pre-treatment with the study drug two hours before stimulation reduced the release of TNF-α and IL-8 from THP-1 monocytes. No effects on IL-10 release were observed [3].

This observation was supported by the determination of the pro-inflammatory cytokines in the compartments on both sides of the cell layer in the TEER model [3].

Multimodal approach to the treatment of functional gastrointestinal disorders (FGIDs)

Healthy gut barrier function plays a fundamental role in gut homeostasis, resistance to harmful microbial colonisation and resistance to pathogens. Given the high prevalence of FGIDs, there is great interest in effective and tolerable therapies to restore or maintain proper gut barrier function.

The multimodal mode of action of combinations of probiotics, herbal extracts and micronutrients can provide a fast-acting treatment option for FGIDs as well as a good alternative to a single probiotic [3–5]. Future studies based on this principle will clarify whether these combinations could be superior to a single strain of probiotics in the treatment of FGIDs. *Chamomilla recutita* extract possesses in vitro anti-inflammatory properties besides an antispasmodic and antidiarrhoeal potential and could thus contribute to the positive effect on typical FGID symptoms [6]. Olive

[1] Personal information from Dr Veronica Cocetta | Post Doc Researcher | University of Padova, Padova | UNIPD | Department of Pharmaceutical and Pharmacological Sciences DSF | Research profile (researchgate.net)

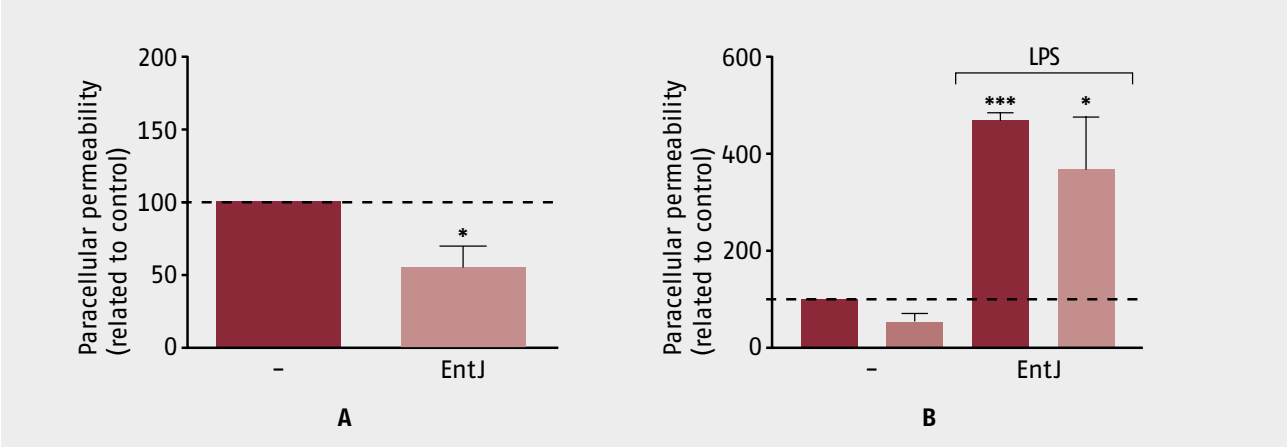

Fig. 1. Effect of pre-treatment with Pegaso® Enterodophilus® Junior (Ent J) on paracellular fluorescein isothyanate flux. A: without induction of inflammatory processes. B: with LPS-induced inflammatory processes. The horizontal dashed line marks the values of the control as 100%. The data are presented as a mean (± SEM) percentage of the basal fluorescence intensity of n = 3 experiments. *$p < 0.05$, ***$p < 0.001$ treatment vs. control [5]

oil polyphenols have anti-inflammatory and antioxidant properties which could have a positive effect on inflammatory mediators as well as on the protection of membrane permeability [7]. Vitamins A and D, as micronutrients, generally protect the mucous membranes and the integrity of the intestinal barrier [5].

Further research is needed to better assess the role of each ingredient in the effective treatment of FGIDs in babies and children.

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