Article

Efficacy of a Product Containing Xyloglucan and Pea Protein on Intestinal Barrier Function in a Partial Restraint Stress Animal Model

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Abstract: Functional abdominal bloating and distension (FABD) are common and frequent symptoms in patients with pre-existing gastrointestinal (GI) disorders. FABD is characterized by recurrent abdominal fullness and bloating. The pathophysiology of FABD is still unclear. However, the plausible mechanisms involved are small intestinal bacterial overgrowth (SIBO), imbalance of gut microbiota, visceral hypersensitivity, intestinal permeability alteration, and disruption of intestinal barrier function. Thus, the creation of a barrier on the wall of the intestine could represent an alternative therapeutic strategy to prevent FABD. This study aimed to investigate the effect of two natural substances, Xyloglucan (XG) and Pea-protein (PP), known for their mucosal-protective properties, in an in vivo model of Partial restraint-stress (PRS). Our results showed that the pre-treatment with a product containing XG and PP in stressed-rats was able to reduce the number of abdominal contractions and visceral hypersensitivity. Moreover, XG and PP were able to reduce intestinal permeability alteration, restoring tight-junctions (TJs) expression and decreased the lactulose–mannitol ratio, a quantitative marker used to measure intestinal permeability, compared to PRS-group. In conclusion, the data obtained revealed that the product containing XG and PP was able to restore the normal intestinal-barrier function; therefore, it could be considered a therapeutic strategy to manage FABD.

Keywords: functional abdominal bloating and distension (FABD); xyloglucan (XG); pea protein (PP); partial restraint stress (PRS); tight junctions (TJs); small intestinal bacterial overgrowth (SIBO)

1. Introduction

Functional abdominal bloating and distension (FABD) are the most common symptoms of functional bowel disorders [1]. FABD is characterized by subjective symptoms of recurrent abdominal fullness, pressure, or a sensation of gas with or without an objective increase in abdominal girth [1]. FABD may be diagnosed as a single entity or may overlap with other functional gastrointestinal (GI) disorders, such as functional constipation (FC), irritable bowel syndrome (IBS), and functional dyspepsia (FD) [2,3]. Abdominal bloating and distension affects about 30% of the general adult population; the majority of FABD-associated symptoms are often recurrent among patients with IBS, especially in women (19%) compared to men (10%) [1]. The pathophysiology of FABD is multifactorial and not completely understood [2].

FABD can be triggered by many factors, such as altered intestinal permeability, gut microbiota alteration, small intestinal bacterial overgrowth (SIBO), visceral hypersensitivity pre-existing gastrointestinal (GI) disorders and anxiety [4–7]. In particular, it has been shown that stress plays a key role in FABD development [1].
The GI tract is particularly vulnerable to stress; it is considered an integral part of the nervous system, made up of a network of nerve fibers and neurons that are influenced by signals from the brain [8]. In fact, it has been demonstrated that the body in response to stressful conditions, suppresses some momentarily non-essential functions, such as digestive functions in favor of those which focus on dealing the stress, resulting in a reduction in digestive secretions, poor absorption of nutrients, increased gut mucosa permeability and abdominal bloating, as observed in patients with FABD [8].

Moreover, many studies suggested that FABD patients exhibited also a disruption of the intestinal barrier function [2,9]. In physiological conditions, the gut mucosa acts as a semipermeable barrier that permits nutrient absorption, regulates immunosurveillance and retains potentially harmful pathogens in the intestinal lumen [9].

The function of the semipermeable barrier is guaranteed by the presence of tight junctions (TJs) which play a key role in the maintenance of gut permeability [10]. TJs are multi-protein complexes, which avoid the diffusion of potentially harmful pathogens through the mucosa, preventing the activation of the inflammatory cascade [11,12]. The disruption of the intestinal barrier function can cause the presence of small intestinal bacterial overgrowth (SIBO), and gas-producing bacteria that adhere to the mucosa, excessively proliferate and produce gas, causing consequently bloating, flatulence and abdominal distension [4,13,14]. Numerous studies have shown that SIBO is closely associated with mucosal inflammation, food overfermentation, intestine permeability alteration, and malabsorption [15,16]. This overgrowth bacterial population could affect the intestinal barrier through a direct bacterial injury or immune system activation resulting in the release of several inflammatory mediators including cytokines, such as interleukin-1β and interleukin-6 [17,18]. Furthermore, it has been demonstrated that the imbalance between intestinal symbiotic bacteria and pathogenic bacteria could alter the mechanical barrier function of the intestinal mucosa, modifying consequently also TJs expression, such as zonula occludens-1, occludin, etc. [16,19].

Moreover, a disruption of the intestinal barrier function can induce altered nutrient sensing that creates neuro-signals causing abdominal distension and recurrent pain [13]. Visceral hypersensitivity is a multifactorial process described as the increased perception of pain in visceral organs that may occur within the peripheral or central nervous system and plays a crucial role in the etiology of abdominal bloating and distension [6,20]. Currently, several agents are available for FABD treatment, such as antispasmodics, antibiotics, e.g., rifaximin, anti-foaming agents, e.g., simethicone and probiotics to reduce the frequency and severity of abdominal distension and bloating [21,22]. However, the use of long-term pharmacological treatments for FABD can often trigger a worsening of the patient’s clinical condition, causing nausea, increased abdominal bloating and allergic reactions [23].

Therefore, great interest has been invested in the use of natural substances for FABD treatment due to a lower possibility of developing side effects compared to synthetic drugs and increasing tolerability [24]. Taking into account the different mechanisms involved in the etiology of bloating and abdominal distension, it is very important to develop alternative natural treatments able to act on visceral hypersensitivity and to prevent the disruption of intestinal barrier function. In this context, the beneficial proprieties of two natural substances, Xyloglucan (XG) and Pea Protein (PP) were evaluated. XG is hemicellulose extracted from the seeds of Tamarindus indica able to restore intestinal barrier function with mucosal-protective properties [25,26]. Recently, XG was approved in Europe as a functional molecule for the restoration of barrier intestinal integrity thanks to its mucosal-protective properties [25]. PP is a protein obtained from Pisum sativum, an herbaceous plant belonging to the Fabaceae family. The pea Pisum sativum is rich in proteins and dietary fiber and contributes considerably to the total dietary protein requirements; PP possesses an extraordinary aminoacidic profile conferring to PP’s favorable health properties and its film-forming features [27]. Given the health-promoting properties, the combined action of XG and PP could maximize the efficacy of the mucosal-protective barrier [28] in the intestine and could be considered an alternative strategy to maintain the normal intestinal barrier.
function, restoring the functionality of TJs and preventing SIBO. Considering the important role that stress plays in the development of FABD, various acute stress animal models have been developed to better understand FABD pathophysiology. Such models include the Partial Restraint Stress (PRS) model [29–31] resulting in a pathophysiological condition similar to FABD.

Therefore, the aim of this study was to investigate the efficacy of a product containing XG and PP to prevent functional abdominal bloating and distension (FABD) in an in vivo model of Partial restraint stress (PRS), a moderate stress procedure to assess the visceralosomatic response.

2. Results
2.1. Effect of XG and PP on Partial Stress-Induced Visceral Hypersensitivity

An abnormal increase of intraluminal contents and visceral hypersensitivity can trigger FABD development [4]. Therefore, in this study, we investigated whether XG + PP was able to prevent visceral hypersensitivity symptoms generated by acute stress. Our results showed that PRS increased the number of abdominal contractions in response to colorectal distension in a volume-dependent manner, reflecting a significant increase in visceral hypersensitivity. Whereas, the pre-treatment with a product containing XG and PP prevented stress-induced visceral hypersensitivity compared to stressed rats treated with saline (Figure 1A). Additionally, our results showed that abdominal contractions were significantly higher in stressed rats compared to the control group; however, stressed rats pre-treated with XG + PP showed a significantly lower response to abdominal contraction (Figure 1B).

![Figure 1. Effect of xyloglucan (XG) + pea protein (PP) on visceral hypersensitivity. Partial restraint stress (PRS) increased the number of abdominal contractions in response to colorectal distension in a volume-dependent manner, reflecting a significant increase in visceral hypersensitivity. However, the pre-treatment with XG + PP prevented stress-induced visceral hypersensitivity compared to stressed rats treated with saline (A). Additionally, abdominal contractions are significantly higher in stressed rats compared to control group; whereas, rats pre-treated with XG + PP showed a significantly lower response to abdominal contraction compared to stressed rats treated with saline (B). The error bars shown in each figure represents the standard deviation Data are representative of at least three independent experiments; (A) *** p < 0.001 vs. Control; ### p < 0.001 vs. PRS; Control vs. Control + XG + PP: p > 0.99; (B) *** p < 0.001 vs. Control; ooo p < 0.001 vs. Control + XG + PP; # p < 0.05 vs. PRS; Control vs. Control + XG + PP: p > 0.99.

2.2. Effect of XG and PP on Gut Permeability

Intestinal permeability and integrity can be measured by the evaluation of passive absorption of monosaccharides [32]. Therefore, in this study, we decided to detect Lactulose/Mannitol (Lac/Man) ratio to assess gut permeability. Our results demonstrated that rats subjected to PRS showed an increased gut permeability as observed by the increased Lactulose/Mannitol (Lac/Man) ratio (Figure 2). However, stressed rats pre-treated with a product containing XG and PP showed a significant reduction of the Lac/Man ratio as shown in Figure 2. Thus, the product containing XG and PP significantly reduced the alteration of gut permeability in stressed rats, restoring the normal intestinal permeability.
Intestinal permeability and integrity can be measured by the evaluation of passive absorption of monosaccharides [32]. Therefore, in this study, we decided to detect Lactulose/Mannitol (Lac/Man) ratio.

2.3. Effect of XG and PP on TJs Expression

FABD, triggered by stressful conditions, is often accompanied by intestinal permeability alteration [5]. Thus, in this study, we decided to investigate TJs expression, in particular claudin-1, zonula occludens-1 (ZO1) and occludin, which are indispensable for normal gut barrier function. Our results revealed a significant expression of claudin-1, ZO-1 and occludin in control groups, while a significant decrease of their expression was observed in all intestinal tracts from PRS groups (Figures 3–5). However, the pre-treatment with XG and PP significantly restored claudin-1, ZO-1 and occludin expression almost to basal level (Figures 3–5). Additionally, we investigated E-cadherin expression, a major component of adherens junctions; it has been shown that an increased expression of E-cadherin in the small intestine and colon is linked to an altered intestinal homeostasis and barrier function, promoting gut permeability alteration [33]. Therefore, our results showed that PRS stress induced a significant increase of E-cadherin expression compared to the control group; however, the pre-treatment with XG and PP was able to reduce the E-cadherin positive staining in all intestine tracts (Figure 6).

2.4. Effect of XG and PP on IL-1β and IL-6 Levels

Studies have shown that FABD is related to an inflammatory state of the intestinal mucosa [34,35]. It has been suggested that abdominal pain, diarrhea, and flatulence may be associated with an excessive inflammatory response in the intestinal mucosa, contributing to the release of cytokines, such as IL-1β and IL-6 [18,34,35]. Therefore, in this study, we decided to detect the levels of IL-1β and IL-6 in colon samples by ELISA assay. Our results showed that the PRS group was characterized by an increase of IL-1β and IL-6 levels compared to the control group; however, the pre-treatment with XG and PP was able to reduce their levels as shown in Figure 7A,B.
Figure 3. Effect of xyloglucan (XG) + pea protein (PP) on claudin-1 expression. A significant reduction in claudin-1 positive cells was observed in the small and large intestine of stressed rats (C,G,K) compared to control groups (A,E,I,B,F,J) while the pre-treatment with XG and PP significantly restored claudin-1 expression, particularly in the ileum and colon (D,H,L). The error bars shown in each figure represents the standard deviation. Data are representative of at least three independent experiments; (M) *** \( p < 0.001 \) vs. Control; °°° \( p < 0.001 \) vs. Control + XG + PP; # \( p < 0.05 \) vs. PRS and ### \( p < 0.001 \) vs. PRS; Jejenum: Control vs. Control + XG + PP: \( p = 0.06 \); Ileum: Control vs. Control + XG + PP: \( p = 0.12 \); Colon: Control vs. Control + XG + PP: \( p = 0.05 \).
Figure 4. Effect of xyloglucan (XG) + pea protein (XG + PP) on ZO-1 expression. A significant reduction in ZO-1 positive cells was observed in the small and large intestine of stressed rats (C,G,K) compared to control groups (A,E,I,B,F,J), while the pre-treatment with XG and PP significantly restored ZO-1 in the intestine tract (D,H,L). The error bars shown in each figure represents the standard deviation. Data are representative of at least three independent experiment; (M) *** p < 0.001 vs. Control; ○○○ p < 0.001 vs. Control + XG + PP; ### p < 0.001 vs. PRS; Jejenum: Control vs. Control + XG + PP: p = 0.06; Ileum: Control vs. Control + XG + PP: p = 0.15; Colon: Control vs. Control + XG + PP: p = 0.06.
Figure 5. Effect of xyloglucan (XG) + pea protein (XG + PP) on occludin expression. A significant reduction in occludin positive cells was observed in the small and large intestine of stressed rats (C, G, K) compared to control groups (A, E, I, B, F, J), while the pre-treatment with XG and PP significantly restored occludin expression, particularly in the small intestine (D, H, L). The error bars shown in each figure represents the standard deviation. Data are representative of at least three independent experiments. (M) *** p < 0.001 vs. Control; °°° p < 0.001 vs. Control + XG + PP; # p < 0.05 vs. PRS and ### p < 0.001 vs. PRS; Jejenum: Control vs. Control + XG + PP: p = 0.06; Ileum: Control vs. Control + XG + PP: p = 0.23; Colon: Control vs. Control + XG + PP: p = 0.07.
**Figure 6.** Effect of xyloglucan (XG) + pea protein (XG + PP) on e-cadherin expression. A significant increase in e-cadherin positive cells was observed in the small and large intestine of stressed rats (C,G,K) compared to control groups (A,E,I,B,F,J), while the pre-treatment with XG and PP significantly reduced its expression, particularly in the small intestine (D,H,L). The error bars shown in each figure represents the standard deviation. Data are representative of at least three independent experiments. (M) *** p < 0.001 vs. Control; °°° p < 0.001 vs. Control + XG + PP; ## p < 0.01 vs. PRS. Jejenum: Control vs. Control + XG + PP: p > 0.99; Ileum: Control vs. Control + XG + PP: p = 0.64; Colon: Control vs. Control + XG + PP: p = 0.49.
2.4. Effect of XG and PP on IL-1β and IL-6 Levels

Studies have shown that FABD is related to an inflammatory state of the intestinal mucosa [8]. Therefore, the use of natural substances capable of creating a barrier on the wall of the intestine could represent an alternative and valid strategy to prevent FABD and restore an important aspect of FABD and may be central to its clinical manifestations [2].

In particular, it has been shown that stress plays a key role in FABD development [4]. Stressful conditions can negatively influence various GI functions, resulting in poor nutrient absorption, abdominal bloating and distension, and impaired gut mucosa function [8]. In a physiological state, the gut mucosa plays a key role in the maintenance of intestinal permeability acting as a semi-permeable barrier that regulates the absorption of nutrients and avoids the passage of substances or pathogens potentially harmful to the intestinal lumen [37]. Moreover, intestinal permeability is regulated by the presence of TJs, multiprotein complexes which control ion, water and solute diffusion, limiting the passage of permeability acting as a semi-permeable barrier that regulates the absorption of nutrients and avoids the passage of substances or pathogens potentially harmful to the intestinal lumen [37]. Moreover, intestinal permeability is regulated by the presence of TJs, multiprotein complexes which control ion, water and solute diffusion, limiting the passage of substances or pathogens potentially harmful to the intestinal lumen [37].

3. Discussion

Functional abdominal bloating and distension (FABD) are common gastrointestinal disorders, such as irritable bowel syndrome (IBS) and functional dyspepsia (FD) [4]. Abdominal bloating is a common symptom defined as a subjective feeling of abdominal pressure regardless of the presence of objective abdominal distension [13]. While FABD has been characterized by a marked disruption of the intestinal barrier function [4], its pathophysiology is still unclear [11]. The plausible mechanisms that may lead to the onset of FABD include small intestinal bacterial overgrowth (SIBO), imbalance of gut microbiota, food intolerance, visceral hypersensitivity and abnormal viscerosomatic responses [5,9,36].

Thus, the disruption of intestinal barrier function and stressful conditions represent an important aspect of FABD and may be central to its clinical manifestations [2].

Therapeutic options for FABD symptomatic treatment include dietary changes, probiotics, antibiotics, prokinetic agents, antispasmodics and neuromodulators [21,22,42].

Despite various symptomatic treatments for FABD, actually no agent is able to act specifically for the treatment of abdominal bloating. Furthermore, long-term drug therapies for FABD treatment can often trigger allergic reactions and various side effects in the GI tract, resulting in a worsening of the patient’s clinical condition [24].

In the last decade, more attention has been given to the efficacy of natural substances for FABD treatment, such as peppermint oil [43] due to its higher tolerability and lower possibility of developing side effects compared to synthetic drugs [24].

Therefore, the use of natural substances capable of creating a barrier on the wall of the intestine could represent an alternative and valid strategy to prevent FABD and restore

![Figure 7](image-url)

**Figure 7.** Effect of xyloglucan (XG) + pea protein (PP) on IL-1β and IL-6 levels. ELISA assay revealed that IL-1β and IL-6 levels are significantly elevated in PRS group compared to control group; however, the pre-treatment with XG and PP reduced their levels. The error bars shown in each figure represents the standard deviation. Data are representative of at least three independent experiments. (A) *** p < 0.001 vs. Control; ** p < 0.001 vs. Control + XG + PP; ### p < 0.001 vs. PRS; Control vs. Control + XG + PP: p > 0.99; (B) *** p < 0.001 vs. Control; ** p < 0.001 vs. Control + XG + PP; ### p < 0.001 vs. PRS, Control vs. Control + XG + PP: p > 0.99.
intestinal permeability compared to the most commonly used synthetic drugs. In particular, great attention was focused on the beneficial properties of two natural substances, XG and PP [44,45]. Previous studies have demonstrated that XG and PP are functional food ingredients with a high safety profile [46,47]. XG and PP are able to form a bioprotective film on the intestinal mucosa that prevent contact with potentially harmful pathogens that characterized many pathological conditions, such as SIBO [6,46]; therefore, XG and PP could be considered a good alternative to mechanically restore the intestinal barrier function thanks to their muco-protective properties.

Furthermore, considering the key role of stress in FABD onset, several acute stress animal models have been developed to mimic FABD features, such as the PRS model; however, none of the available models are able to entirely reproduce stress responses [29–31]. In animals, the PRS model is known to increase visceral sensitivity, colorectal distension, and intestinal barrier function alterations, resulting in a physiological and symptomatic condition similar to FABD [30,31].

Therefore, the aim of this study was to investigate the efficacy of a product containing XG and PP to prevent abdominal bloating and distension in an in vivo model of Partial Restraint Stress (PRS).

Firstly, in this study, we decided to evaluate visceral hypersensitivity by evaluating the number of abdominal contractions in response to colorectal distension. Visceral hypersensitivity is described as an increased perception of pain originating from the viscera [48]. It is a hallmark feature of FABD and is currently regarded as the main factor underlying abdominal pain in patients with functional GI disorders [40,49]. In this context, our results demonstrated that the PRS group was characterized by an increase in the number of abdominal contractions in response to colorectal distension, in a volume-dependent manner, highlighting a significant increase of visceral hypersensitivity. However, the pre-treatment with the product containing XG and PP prevented stress-induced visceral hypersensitivity by reducing the number of abdominal contractions compared to the PRS group. Additionally, we decided to investigate the response to colonic distension at all distending volumes after oral administration of XG and PP. Our results showed that abdominal contractions were significantly higher in stressed rats compared to the control group. Whereas, the group pre-treated with XG + PP showed a significantly lower response to abdominal contraction compared to the PRS group treated with saline, confirming that XG + PP was able to prevent stress-induced visceral hypersensitivity.

An essential role of the intestinal epithelium is the regulation of ions and solutes diffusion through a semipermeable barrier for maintenance of gut permeability [50,51]. Intestinal permeability can increase physiologically in response to luminal nutrients, pathogens, or stressful conditions [52]. Thus, an impaired intestinal barrier is associated with a number of clinical conditions, both intestinal and systemic, indicated by the presence of excessive concentrations of solutes in urine after renal excretion [50,53,54].

Therefore, to evaluate the ability of XG + PP to regulate gut permeability we detected the Lac/Man ratio as an index of intestinal permeability [54]. Our results demonstrated that the PRS group was characterized by an increase in the Lac/Man ratio compared to the control group. However, the pre-treatment with XG and PP significantly reduced the Lac/Man ratio, confirming that the pre-treatment with XG and PP, thanks to its mucin-mimetic effect, was able to significantly reduce intestinal permeability alteration.

Moreover, FABD is characterized by a disruption of intestinal barrier function that is generally guaranteed by the presence of TJ [11]. Changes in the expression, localization, or phosphorylation status of TJ can alter intestinal barrier permeability, which may allow the uptake of toxins or antigens [11]. The presence of xylose and galactoxylose ramifications in the structure of XG gives the product a “mucin-like” molecular structure, conferring excellent mucoadhesive properties [46,59]; this allows the XG formulations to form a bioprotective barrier capable to preserve TJ component, paracellular flow, to avoid pathogenic bacteria adherence and to maintain intestinal permeability as demonstrated in various studies [46,55,56]. The beneficial effects of film-forming agents, such as XG and also PP have
also been demonstrated in patients with irritable bowel syndrome [27,44,45]. Thus, based on the muco-adhesive and filming properties of XG and PP, we decided to evaluate their ability to restore intestinal permeability, by evaluating TJs expression, especially claudin-1, ZO-1 and occludin, multiprotein complexes which regulate the intestinal permeability by allowing the passage of water, ions, and solutes through pores [11]. Our results showed that the PRS group was characterized by a decrease of claudin-1, ZO-1 and occludin expression compared to the control group, while the pre-treatment with XG and PP was able to restore their expression almost to basal levels thanks to their muco-adhesive and filming properties exercise on the barrier intestinal. Additionally, we decided to evaluate the role of E-cadherin, a key component of adherens junctions that not only mediates adhesion between cells but also regulates the linkage of adhesive complexes to the underlying actomyosin cytoskeleton [33,57]. Many studies demonstrated an increase of E-cadherin level in response to stressful conditions in the intestinal tract promoting gut permeability alteration [33,58]. In this study, we observed a significant increase of E-cadherin expression in the PRS group, particularly in ileum sections, compared to control group. However, the pre-treatment XG + PP was able to reduce E-cadherin expression in all intestinal tracts, confirming the ability of XG + PP to restore intestinal permeability.

Furthermore, it has been demonstrated that the disruption of the intestinal barrier function is associated with SIBO and mucosal inflammation [34,35]. Studies suggested that abdominal pain, diarrhea, and flatulence may be associated with an excessive inflammatory response in the intestinal mucosa [35]. The inflammatory response in the intestinal mucosa contributes to the release of numerous inflammatory mediators, such as cytokines, which promote FABD symptoms progression [35]. Thus, considering the link between FABD and inflammation, it is also important to understand the impact on SIBO and/or the interaction of these two ingredients. Therefore, we decided to evaluate two inflammatory markers, such as IL-1β and IL-6 [18,34]. Our results showed that the PRS group was characterized by an increase of IL-1β and IL-6 levels compared to the control group; however, the pre-treatment with XG and PP was able to prevent cytokine release.

In summary, these results revealed the therapeutics benefits of the product containing XG and PP to reduce visceral hypersensitivity and intestinal permeability alteration associated with stressful conditions in an in vivo model of PRS. Despite the promising results obtained in this study, further studies in humans should be performed to attain a comprehensive understanding of FABD onset and progression, as well as to evaluate the efficacy of XG and PP in other factors involved in FABD development, such as its influence on gut microbiota alteration. Moreover, it would be interesting to follow the evolution of SIBO in FABD development in humans through novel clinical studies.

4. Material and Methods

4.1. Animals

Sprague–Dawley rats female (Harlan, Milan, Italy) were housed in a controlled environment (22 ± 2 °C, 55 ± 15% relative humidity, 12 h light/dark cycle). After one week of acclimation, rats were fed with a standard diet and water ad libitum. Animal experiments were in compliance with Italian regulations on the protection of animals used for experimental and other scientific purposes (DM 116192) as well as EU regulations (OJ of EC L 358/1 12/18/1986). The animals used for this study were randomly selected from those suitable, available at that time.

4.2. Partial Restraint Stress Induction

Partial restraint stress (PRS), a relatively mild stress, was performed as previously described by Bueno et al. [59]. Briefly, animals were anesthetized with isoflurane, and their freeholders, upper forelimbs and thoracic trunk were wrapped in a confining harness of paper tape in order to restrict, but not prevent, their body movements. Then, rats were placed in their home cage for 2 h. Animals were pretreated by oral gavage tube twice daily with 200 mg/kg of a product containing 140 mg of Pea protein (PP) and 60 mg of
Xyloglucan (XG) for 5 days. Previous studies were performed to define the best ratio and dosage of the two ingredients. Different ratios of xyloglucan and pea protein were tested using an enteritis model (unpublished data). The setting consisted of oral administration of test compounds before IP injection of LPS from E.coli. LPS administration induced a strong increase in jejunal permeability which was significantly prevented by the oral treatment of xyloglucan and pea protein. The highest protective effect was obtained using the xyloglucan and pea protein ratio (30:70).

The vehicle used was phosphate-buffered saline (PBS) administered by an oral gavage feeding tube. PRS was performed between 10:00 and 12:00 a.m., followed by colorectal distension (CRD) for 5 min. The animals were sacrificed at the end of PRS/CRD-induced stress.

Experimental groups:
- Group 1. Control. Rats received vehicle orally (n = 6).
- Group 2. Control + XG + PP. Rats received XG + PP orally daily (n = 6).
- Group 3. PRS. Rats were subjected to PRS and to CRD (n = 12).
- Group 4. PRS + XG+PP. Rats received XG+PP orally, twice a day, and then were subjected to PRS and to CRD (n = 12).

The lactulose/mannitol (Lac/Man) test was performed in another set of experiments.

4.3. Colorectal Distension to Evaluate Visceral Sensitivity

After completion of the 2 h PRS period, colonic sensitivity was assessed by measuring the intra-colonic pressure required to induce a behavioral response during colonic distension due to the inflation of a balloon introduced in the colon as previously described by Boulete et al. [60]. During the acclimation sessions, rats were placed in plastic tunnels where they could move but not escape. Prior to the CRD procedure, a balloon (latex condom) was inserted into the rectum of conscious rats until the base of the balloon was at the anus (4 cm insertion). The tube was fixed at the base of the tail and rats were allowed to recover for 30 min. The balloon was then connected to a barostat and inflated progressively from 0–60 mmHg in 15 mmHg steps. Each step of inflation lasted five min. Responses to applied CRD pressure levels were measured with electromyographic recordings during the five-min interval and data are expressed as contractions/five min.

4.4. Lactulose/Mannitol (Lac/Man) Test to Evaluate Gut Permeability

The lactulose and mannitol (Lac/Man) test was performed using standard doses of the sugars to detect gut permeability as previously described [32]. Lactulose and mannitol were administered simultaneously to the rats by gavage at concentrations of 5 mg of lactulose and 12.5 mg of mannitol for each rat. After gavage, the urine of rats was collected in full over 24 h at the end of PRS-CRD-induced stress for sugar analyses and stored at −80 °C for HPLC analysis. To collect urine, rats were individually isolated in the metabolic cages. The urinary rates of both sugars were calculated as a percentage of the ingested dose in mg.

4.5. Immunofluorescence Assay for Tight Junctions (TJs)

Immunofluorescence staining (IF) was performed as previously described by Campolo et al. [61]. Jejunum, ileum and colon samples were collected and processed for immunofluorescence staining. Tissue sections of 7 µm were incubated with the following primary antibodies at 37 °C overnight: anti-Claudin1 (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Zonula Occludens1 (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-E-cadherin (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Occludin (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Then, tissue sections were washed with phosphate buffered saline (PBS) and incubated with the following secondary antibody, anti-rabbit Alexa Fluor-594 antibody (1:1000 in PBS, v/v, Molecular Probes, Altrincham, UK) and anti-mouse Alexa Fluor-488 antibody (1:1000 v/v, Molecular Probes, Altrincham, UK) for 1 h at 37 °C. For nuclear staining, 4′,6′-diamidino-2-
phenylindole (DAPI; Hoechst, Frankfurt, Germany) (2 µg/mL) in PBS was added. Sections were observed and photographed at 40× magnification using a Leica DM2000 microscope.

4.6. ELISA Assay for IL-1β and IL-6

The levels of IL-1β and IL-6 were performed as previously described [62] The samples were homogenized and centrifuged at 14,000× g for 10 min at 4 °C; IL-1β and IL-6 were measured by ELISA kits according to the manufacturer’s instructions (Rat IL-1β ELISA kit cat. No. MBS825017 MyBiosource; San Diego, CA, USA; Rat IL-6 ELISA kit cat. No. MBS175908 MyBiosource, San Diego, CA, USA).

4.7. Materials

The product containing XG and PP was kindly provided by DEVINTEC SAGL (Lugano, Switzerland). All chemicals were obtained from the highest grade of commercial sources. XG was obtained from Tamarind seed gum. Tamarind seed gum has Chemical Abstracts Service (CAS) registry number 39386-78-2 is used as a thickener, stabilizer, or gelling agent for various foods (percentage of 99.99%). Pea protein is extracted from the yellow pea, a natural and gluten free raw material, with a composition of proteins 88 +/− 2%; free from any harmful or toxic substances. The manufacturing process of Pea protein includes an initial step of sieving to establish the size distribution of the “particles”, followed by destoner, de-hulling, and milling. The next phase includes solubilization, decantation and lastly thermal treatment to obtain the final product.

4.8. Statistical Analysis

All data were expressed as the mean ± SEM. Statistical analyses were performed using PRISM 7 version 5.0 (SPSS Inc., Chicago, IL, USA). Data at different time points were analyzed using a two-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test. Other data were analyzed using one-way ANOVA followed by a Bonferroni post-hoc test. The data for immunofluorescence staining (IF) were analyzed using t-test analysis. p-value < 0.05 was considered as statistically significant. Lac/Man ratio data were analyzed using one-way ANOVA followed by the Dunnet post-hoc test. p-value < 0.05 was considered as statistically significant.

5. Conclusions

In conclusion, the product containing XG and PP demonstrated the ability to significantly reduce stressed-induced visceral hypersensitivity and colorectal distension in stressed rats. Moreover, the pre-treatment with XG and PP reduced the alteration of gut permeability, restoring the normal intestinal permeability. Given our findings, we believe the product containing XG and PP could be considered an effective therapeutic strategy to treat FABD.

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