Diarrhea-predominant irritable bowel syndrome (IBS-D) is a chronic functional gastrointestinal disorder defined by clinical symptoms such as diarrhea, abdominal pain, abdominal distension, and recurring episodes, usually occurring without notable changes in organ structure. Multiple contributing factors for IBS-D include neurotransmitter disorders, inflammation, intestinal barrier dysfunction, intestinal microbiota disruptions, stress, genetic factors, and others. The pomegranate peel extract (PPE), which is made from the peel of the pomegranate (Punica granatum), a member of the Punicaceae family, has strong anti-inflammatory, antibacterial, antioxidant, anticancer, and anti-diarrheal properties. These effects are attributed to its high concentrations of polyphenols, tannins, and anthocyanins. In this study, the Chinese authors investigated the underlying mechanisms and therapeutic effects of pomegranate peel extract in a rat model of irritable bowel syndrome.

Intestinal epithelial cells form a protective barrier and are crucial for initiating an appropriate mucosal immune response after infection or injury. An increased goblet cell number upregulates mucin expression and facilitates the repair and functional stabilization of the damaged intestinal mucosal barrier. In contrast, a reduced goblet cell number leads to thinning of the mucosal layer, promoting intestinal inflammation. The inflammatory response and oxidative stress are among the major factors contributing to the pathogenesis

of intestinal diseases. Mitogen-activated protein kinases (MAPK), including c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38 MAPK), and extracellular signal-regulated kinase (ERK), are sequentially activated and regulate a wide range of important pathological processes, including intestinal inflammation. Therefore, modulation of the NF-κB and MAPK signaling pathways may represent a promising strategy for managing IBS-D.

Previous studies have shown that PPE can attenuate tissue damage in *Citrobacter*-induced colitis in mice, enhance bacterial clearance by promoting phagocytosis *via* activation of the NF-κB and MAPK signaling pathways, mitigate the changes induced by high-fat diet by modulating intestinal flora, and scavenge hydrogen peroxide and free radicals, thereby preventing oxidative damage to cells. In addition, the components of pomegranate peel, ellagic tannins, ellagic acid, and urolithins can contribute to the functional stabilization of the intestinal barrier. These findings underline the potential of PPE in the treatment of intestinal diseases.

About the study

Animal model of IBS-D was established in 5- to 6- week-old male rats. They were exposed to cold water stress daily for one week, then received 3 g/kg senna extract and were subjected to daily restraint stress for two weeks. PPE was administered at a dose of 200 mg/kg or 400 mg/kg, twice daily for 5 days. Blood samples were collected before and 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, and 48 h after the last administration.

24 rats with IBS-D were randomly divided into four groups (n = 6 per group): the model group (administered sterile water), the pinaverium bromide (PVB) group, the PPE high-dose group (400 mg/kg), and the PPE low-dose group (200 mg/kg). A group of healthy rats (n = 6) served as the control group. The severity of visceral pain in response to colorectal distension was assessed using the Abdominal Withdrawal Reflex (AWR) scores. At the end of the experimental period, all rats were anesthetized and euthanized. Fecal, serum, and tissue samples were collected for subsequent analysis. Network pharmacology analysis, serum medicinal chemistry, and transcriptomics were employed to investigate potential downstream signaling pathways. Findings were further validated through molecular docking and Western blot analysis. The chemical composition of PPE was assessed by ultra-performance liquid chromatography-tandem mass spectrometry.

Results

During the characterization of the main components of PPE extract, a total of 189 components were identified, including 35 flavonoids, 17 organic acids, 12 amino acids, 11 fatty acids, 11 terpenoids, 10 phenols, 8 lignans, 7 glycosides, 7 amides, 6 esters, 4 nucleosides, 4 vitamins, 3 alkaloids, 3 hormones, 3 amino alcohols, 3 aromatic aldehydes, and 43 others. According to the authors, ellagic acid, gallic acid, quercitrin, punicalin, ursolic acid, and epicatechin could have a key therapeutic role.

In the IBS-D model established by the combination of a high-fat diet, senna administration, and restraint stress, PPE treatment significantly alleviated diarrhea, as shown by the reduction in weight loss, AWR score, and water content in the stool. The PPE alleviated the IBS-D symptoms *via* inflammatory cytokine level regulation and antioxidant capacity enhancement. PPE treatment decreased levels of inflammatory cytokines (MPO, IL-6, and IL-1β) in both serum and colon tissue and increased levels of the anti-inflammatory IL-10. In addition, PPE treatment modulated indices of oxidative stress, such as MDA, T-AOC, GSH-Px, and CAT, showing enhanced self-protective mechanisms.

Transmission electron microscopy of colon tissue samples revealed a significant role of PPE in maintaining the integrity of the intestinal barrier, as evidenced by increased goblet cell numbers, increased microvilli density, and increased expression of the tight junction proteins occludin, claudin1, and ZO-1. These observations were further corroborated at the mRNA level, suggesting that PPE modulates the expression of intestinal barrier proteins and improves intestinal permeability.

Transcriptomic analysis showed that PPE significantly altered gene expression in IBS-D. In rats treated with PPE, 1821 genes were upregulated and 2299 genes downregulated compared to the model group treated with sterile water. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis further identified the MAPK and NF-κB signaling pathways as key mechanisms underlying the therapeutic effects of PPE in IBS-D. Differential gene expression analysis within the MAPK and NF-κB signaling pathways showed that key genes, including Mapk10, Mapk8ip2, Gadd45g, Fos, Map3k8, Cxcl2, Cxcl1, Ptgs2, Gadd45g, and Jun were consistently downregulated after PPE treatment.

The binding energies of nine major active components of PPE, including ellagic acid, epicatechin, quercitrin, punicalin, and chlorogenic acid, were below −7 kcal/mol with all nine MAPK proteins. The molecular docking of nine PPE components with the P65 and IκB

proteins within the NF-κB signaling pathway also yielded docking scores below −5 kcal/mol. These results indicate that the primary components of PPE can effectively interact with the MAPK and NF-κB signaling pathways.

The Western blot analysis showed a significant upregulation of phosphorylated JNK, ERK, and P38 in the IBS-D rats, indicating a state of oxidative stress. The administration of PPE reversed this trend, resulting in a significant decrease in the expression of these proteins. The mRNA levels of key proteins within the MAPK signaling pathways significantly decreased after the PPE administration. NF-κB signaling pathway demonstrated a similar trend.

Conclusion

This study demonstrated that PPE administration in a rat model of IBS-D alleviated intestinal symptoms, attenuated tissue damage, and mitigated the inflammatory response. The MAPK and NF-κB signaling pathways were key mechanisms underlying the therapeutic effects of pomegranate peel extract in irritable bowel syndrome.

The authors concluded that these findings provided a theoretical basis and underlined the potential of PPE in the treatment of intestinal diseases.

This article was published in Nutrients.

Journal Reference

Zhang, Y, Huang, S, Zhang S. et al. Pomegranate Peel Extract Mitigates Diarrhea-Predominant Irritable Bowel Syndromes via MAPK and NF-κB Pathway Modulation in Rats. Nutrients 2024, 16, 3854. https://doi.org/10.3390/nu16223854