Letter to the Editor

Bitter Melon Powder Protects against Obesity-associated Fatty Liver Disease by Improving Colonic Microenvironment in Rats with High-fat Diet-induced Obesity*

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This study explored how bitter melon powder (BMP) alters the colonic microenvironment during the development of obesity-associated fatty liver in rats. We observed that BMP effectively inhibited the body weight gain and lipid accumulation in the liver, ameliorated glucose intolerance, and increased the colon weight after an 8-week treatment compared to that in the high-fat diet (HFD) group. BMP significantly decreased fecal water toxicity towards HT-29 cells, as revealed by the cell counting kit (CCK)-8 assay results, and the mRNA expression of Toll-like receptor 4 (TLR4) in colon mucosa. Additionally, gut permeability in the BMP group was restored to normal levels. Finally, BMP alleviated the inflammatory state of the rat colon mucosa and liver tissues as well as the systemic inflammation.

Recently, obesity and its metabolic complications have emerged as major health concerns worldwide. Studies have demonstrated that the gut plays a fundamental role in the development of chronic metabolic diseases[1]. Nowadays, besides the sequencing of the gut microbiota profile, the detection of fecal water toxicity towards intestinal epithelial cells can indirectly reflect the state of the intestinal flora and the effect of its metabolites on the host[2]. In the gut, the microbial metabolismis mostly responsible for the complex composition of fecal water. Metabolites in the fecal water produced by the gut microbiota could be absorbed into the bloodstream and, subsequently, impair the gut barrier function or enterohepatic circulation. Some of these gut microbiota-derived metabolites positively affect the host, but others exert toxicity, such as cytotoxins and genotoxins. Increased levels of gut-derived endotoxins would activate Toll-like receptor 4 (TLR4) in colon cells, leading to the induction of pro-inflammatory cytokines, which can cause an imbalance in the intestinal barrier function and ultimately activate inflammatory pathways in peripheral organs, such as adipose and liver tissues.

Bitter melon (*Momordica charantia* L.) has been used as a folk medicine for disease prevention, particularly for diabetes and obesity. Related mechanistic studies have found that the anti-obesity effect of bitter melon is linked to its anti-inflammation property[3]. However, in the obese, the exact mechanism of action by which bitter melon protects against the development of obesity-associated fatty liver *via* the inflammatory response remains elusive. Therefore, this study aimed to evaluate the effects of bitter melon powder (BMP) treatment on colonic fecal water toxicity, gut immunity, gut permeability, and metabolic disorders in the liver.

Rats were divided into the following three groups (n = 10 each) and treated as indicated: (a) normal control diet (NCD) and distilled water, (b) HFD and distilled water, and (c) HFD and 400 mg BMP/kg body weight (bw). We conducted oral glucose tolerance tests (OGTT) and measured systemic inflammation indices, triacylglycerol (TG) and total cholesterol (TC) levels in the liver tissues, using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Harvested colon and liver tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into sections of approximately 4 μm, and stained with hematoxylin and eosin (H&E). The cell counting kit (CCK)-8 assay using tetrazolium-8-[2-(2- methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] monosodium salt was performed to determine the cytotoxicity of fecal water towards HT-29 cells. Gut permeability was evaluated by determining the lactulose/mannitol (L/M) excretion

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ratio and mRNA expression levels of tight junction proteins such as zonula occludens-1 (ZO-1) and occludin. The mRNA expression levels of TLR4 and some inflammatory cytokines in the colon mucosa and liver tissues were detected by quantitative real-time polymerase chain reaction (PCR). The primer sequences of all tested genes are listed in Table S1, available in www.besjournal.com.

The data are presented as the means ± standard deviation (SD) and were analyzed using the statistical package for the social sciences (SPSS) IBM Statistics 20 software (SPSS, Inc.). A one-way analysis of variance (ANOVA) with Tukey's range test was performed to identify the differences between independent sample groups. Significant differences were confirmed at $P < 0.05$ at a 95% confidence limit.

The results indicate that BMP decreased the body weight gain and Lee's index without changing the food intake (Table S2, available in www.besjournal.com). Figure S1 (available in www.besjournal.com) shows that BMP improved oral glucose tolerance levels and significantly decreased the area under the curve (AUC, $P < 0.05$). BMP exhibited anti-obesity effects and remarkably alleviated glucose tolerance in HFD-fed rats. Regarding the intestinal integrity, we found that BMP treatment increased both the colon length and weight (Table S2, available in www.besjournal.com), suggesting it exerted a trophic effect on the intestinal epithelial cells. Everard[4] found that both the colon weight and length were markedly increased by prebiotic treatment of genetically obese and diet-induced leptin-resistant mice. Moreover, we demonstrated that BMP consumption significantly changed the colonic crypt length, which could be considered beneficial to the digestion and absorption of food ($P < 0.05$, Figure 1A and 1C).

The metabolites in the colon content produced by fermentation induced by the resident microbiota have some levels of toxicity. Fecal water represents the portion of the colonic contents that directly contacts the colonic epithelial cells and is a good tool for assessing the role of dietary intervention in the colon. Research studies have reported that diets that are high in fat but low in dietary fiber increased fecal water toxicity against colonic cells[5], which is consistent with our present study results (Figure 2). Study outcomes have also indicated that dietary intervention with BMP in obese rats significantly reduced fecal water cytotoxicity to levels similar to that in the normal rats ($P < 0.05$). A considerable number of experiments have shown that different dietary interventions affect the toxicity of fecal water against intestinal epithelial cells. For example, supplementation of a low-fiber diet with konjac glucomannan reduced the toxicity of fecal water and precancerous risk factors for the development of colon cancer, which was associated with changes in β-glucuronidase activities, microflora, and bile acid levels[6]. Studies have mostly shown that the alteration of fecal water toxicity by dietary intervention was linked to the levels of some specific metabolites such as second bile acid, endotoxin, and short-chain fatty acids (SCFAs). We speculated that the changed levels of SCFAs and endotoxin might exert beneficial effects on the fecal water toxicity, based on our previous study[7]. However, the metabolites derived in the gut are extremely complex and therefore, the entire metabolite profile requires further studies.

Long-term exposure of the colonic mucosa to fecal water containing toxic metabolites potentially affects epithelial cell metabolism and barrier function. TLR4 plays an important role in the intestinal inflammatory responses, along with the increased expression and release of pro-inflammatory cytokines. The present study revealed that HFD activated colonic TLR4 expression and upregulated the levels of the inflammatory factors, tumor necrosis factor (TNF)-α, and interleukin (IL)-6, and these effects were efficiently inhibited by BMP (Figure 3A). Related studies have reported that bitter melon inhibited bacterial mutagenesis and aberrant crypt focus formation in the rat colon tissue, and methanolic extracts of bitter melon suppressed colon cancer stem cells by affecting energy homeostasis and autophagy[8]. However, few research studies have reported the effects of bitter melon on the colonic inflammation in the obese.

Study outcomes suggest that TLR4 and some inflammatory factors such as TNF-α and IL-1β could alter tight junctions and increase intestinal permeability. On the other hand, the increased permeability of the colon is postulated to participate in a feedback loop with inflammatory processes. Intercellular tight junctions, including transmembrane proteins (such as occludin and claudins) and junctional complex proteins (such as ZO-1), play an important role in the permeability properties of the gut barrier. Studies suggest that the whole-gut permeability was increased in HFD mice, with a reduction in mRNA expression of tight