Research in Pharmaceutical Sciences, October 2022; 17(5): 558-571
Received: 31-03-2022
Peer Reviewed: 28-05-2022
Revised: 15-06-2022
Accepted: 16-08-2022
Published: 08-09-2022

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Extracted yam bean (*Pachyrhizus erosus* (L.) Urb.) fiber counteracts adiposity, insulin resistance, and inflammation while modulating gut microbiota composition in mice fed with a high-fat diet

Putra Santoso[1,*], Rita Maliza[1], Resti Rahayu[1], Yunita Astrina[1], Firman Syukri[1], and Septalian Maharani[1]

[1]Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra 25163, Indonesia.

Abstract

Background and purpose: Yam bean (*Pachyrhizus erosus*) is a potent medicinal plant exerting therapeutical effects against diseases. However, investigations on the health benefits of its fiber remain limited. This study aimed to investigate the potential of yam bean fiber (YBF) against a high-fat diet (HFD)-induced metabolic diseases, inflammation, and gut dysbiosis.

Experimental approach: Adult male mice were assigned to four groups (8 each), namely a normal diet-fed group (ND), HFD-fed group, and HFD supplemented with YBF groups (HFD + YBF) at a dose of 2.5% and 10%, respectively. Treatments were implemented for ten weeks. Thereafter, indicators of metabolic diseases, oxidative stress, inflammation, and gut microbiota composition were determined.

Findings / Results: A dosage of 10% YBF significantly inhibited excessive body weight gain (2.3 times lower than HFD group) and white adipose tissue (WAT) mass (2.2 times lower than HFD group) while sustaining brown adipose tissue mass. YBF prevented malondialdehyde elevation, catalase activity reduction, and expression of the interleukin-6 increment (2.7 times lower than the HFD group) within the WAT. Furthermore, YBF sustained normoglycaemia, glucose tolerance, and insulin sensitivity while precluding hyperinsulinemia. YBF modulated the gut microbiota community by increasing health-promoting microbiota including *Lactobacillus reuteri*, *L. johnsonii,* and inhibiting a pathogenic *Mucispirillum* sp. YBF prevented histopathology and inflammation of the colon.

Conclusion and implications: YBF at the dose of 10% is proved to be useful in the prevention of diet-induced metabolic diseases, microbiota dysbiosis, and inflammation. Hence, YBF is recommended as a potential natural-based remedy to diminish the detrimental effects of high-fat foods.

Keywords: Hyperinsulinemia; Inflammation; Interleukin-6; Metabolic diseases; *Mucispirillum* sp.; White adipose tissue.

INTRODUCTION

Metabolic dysregulation and various health problems such as adiposity, dyslipidemia, insulin resistance, inflammation, and gut microbiota dysbiosis have been linked to the chronic and excessive consumption of a high-fat diet (HFD) (1-3). Globally, these diseases have become more prevalent among life-threatening health issues (4). Therefore, to improve the longevity and quality of human lives, it is necessary to formulate affordable and effective natural-based remedies to counteract HFD-induced metabolic dysregulation.

Yam bean (*Pachyrhizus erosus* (L.) Urb., Fabaceae), one of the prominent and widely distributed agricultural products in subtropical and tropical regions, is often served as a vegetable and medicinal herb (5).

*Corresponding author: P. Santoso
Tel: +62-81374151306, Fax: +62-75171085
Email: putrasantoso@sci.unand.ac.id

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.355213

Previous studies have illustrated the beneficial effects of yam bean extract against metabolic diseases such as diabetes mellitus (6,7) and cardiovascular comorbidities (8). While extensive investigations regarding the health benefits of yam bean tuber dietary fibers (YBF) remain limited, YBF has been reported to exert immunomodulatory effects (9). Research has also shown that YBF inhibited the development of obesity and hyperglycaemia in mice fed with a high-sucrose diet (10) and that dietary fibers may exert various beneficial effects against metabolic dysregulation (11,12). A previous study demonstrated that nano-sized fibers extracted from sugarcane could significantly attenuate lipid deposition in the liver of HFD-fed mice by enhancing the signaling system composing hepatic fibroblast growth factor 21, AMP-activated protein kinase, and insulin (11). Finally, as illustrated in type-2 diabetes mellitus (T2DM) patients, the consumption of a high-fiber snack effectively decreases c-reactive peptide and body mass index levels (13). Whether dietary fiber of yam bean tubers also counteracts detrimental effects caused by HFD, however, remains unclear.

The present study aimed to investigate the potential of YBF in counteracting the development of HFD-induced metabolic diseases, inflammation, and gut microbiota dysbiosis. It was expected that the incorporation of YBF in a murine diet would minimize the detrimental effects of HFD consumption on mice's health.

MATERIALS AND METHODS

Extraction of YBF

Five-month-old yam bean tubers were harvested in the Padang Pariaman District, West Sumatra, Indonesia. These tuber samples were immediately transported to the laboratory where they were sequentially washed with tap water (5×). Thereafter, samples were peeled and ground to a porridge consistency using an electric grinder (Tencan 0.4A XQM, China). Water extraction of fiber from the porridge was achieved using a previously described protocol (9). Briefly, the yam bean porridge was transferred into a jar and soaked in the distilled water (1:4) overnight at 4 °C until the fibers (as supernatant) were clearly separated from the starch (as pellet). Subsequently, the fibers were carefully collected, filtered, and then steamed at 100 °C for 30 min before being dried in the oven at 68 °C for 16 h. The sample was then ground to be fine powder and kept in the sealed sterilized jar until use.

Animal provision and experimental treatments

Male mice (n = 32; seven weeks old; 25-26 g body weight) belonging to the Deutschland, Denken, and Yoken (DDY) strain, were purchased from the Veterinary Monitoring and Investigation Center, Baso, Bukittinggi, West Sumatra, Indonesia. These animals, individually housed in cages, were acclimated for a week under regulated temperature (25.5-26.0 °C), humidity (66-67.5%), and 12/12-h light/dark cycles. During this period, water was provided *ad libitum* and mice were fed a standard rodent diet (RATBIO, Citra Ina Fedmill, Indonesia). After acclimatization, mice were randomly divided into four groups based on diet (n = 8), namely (1) the normal diet (ND) group, (2) the HFD group, and the HFD groups supplemented with (3) 2.5% YBF (HFD + 2.5% YBF) or (4) 10% YBF (HFD + 10% YBF). The diet treatments were conducted for ten weeks. The higher dose of YBF (10%) was considered based on the previous study (12) indicating that 10% of dietary fiber (extracted from bamboo shoots) was effective in counteracting HFD-induced metabolic diseases in mice. The lower dose (2.5%) was selected based on our preliminary study for four weeks of treatment indicating that it was the minimum effective dose of YBF exerting a preventive effect against HFD-induced excessive body weight gain. The ND of RATBIO contained 4% fat, 39.3% carbohydrates, and 20% protein (w/w), while the HFD was prepared by supplementing 79% ND with 21% milkfat (14). To ensure quality and hygiene, the food and water of the respective treatments were changed daily throughout the 10-week study. The protocols of this study have been approved by the Research Ethics Committee of the Medicine Faculty, Andalas University (Approval No. 528/ UN.16.2/ KEP-FK/ 2021).

Measurements of body weight and adipose tissues

Body weight was recorded every two weeks (in the morning between 09:00 and 10:00) using a digital balance (SF-400C, Zhezhong Weighing Apparatus Factory, China). Total body weight gain was calculated as the increased percentage of final body weight *vs* initial body weight (10).

Blood glucose measurements

Blood samples from the tail tips of mice were obtained at 09:00 and glucose levels were measured using an automatic glucometer (AGM-4000Allmedicus, South Korea). At the end of the treatment, mice fasted for 18 h (starting at 18:00) and blood samples were subsequently measured for fasting blood glucose levels (10).

Glucose and insulin tolerance tests

An intraperitoneal glucose tolerance test was performed at the end of the 10-week experiment. Mice fasted for 6 h (from 07:00) before d-glucose (20 g/kg BW; Sigma-Aldrich, USA) was intraperitoneally injected. Blood glucose measurements were subsequently performed at 0, 15, 30, 60, 90, and 120 mins using a glucometer. The area under the curve (AUC) was calculated based on blood glucose values. An intraperitoneal insulin tolerance test using the intraperitoneal insulin injection (0.75 IU/kg BW; human insulin Actrapid, Novo Nordisk, Denmark) was also conducted after mice fasted for 6 h (from 07:00) (2).

Measurements of plasma insulin

At the end of the 10-week treatments, mice (having fasted for 8 h) were euthanized with a lethal dose of ketamine and blood samples were obtained through the sinus orbitalis. Plasma separation was achieved through centrifugation at 3000 rpm for 10 min (at 4 °C). Plasma samples were stored at -80 °C before measurements were taken (2). Plasma insulin levels were subsequently determined using mouse ELISA assay kits (Bioassay Technology Laboratory, Shanghai, China) and absorbance was determined using a microplate ELISA reader (xMark 1681150, Bio-Rad Laboratory Inc., USA).

Tissue weight and histological examination

After euthanization, adipose tissues including epididymal white adipose tissue (eWAT), interscapular brown adipose tissue (iBAT), and colon were gently removed and measured for their mass. Thereafter, all tissue samples were fixed in 10% neutral-buffered formalin for 24 h and processed for histological examination according to previously described protocols (15). Representative histological slides were examined and photographed using a microscope (CX31 Olympus, Japan). The histomorphological and histopathological features of samples were then analyzed using Windows software ImageJ (National Institute of Health, NIH, USA; freely downloaded from https://imagej.nih.gov/ij/download.html).

Measurements of malondialdehyde levels and catalase activity in the WAT

Fresh WAT samples of 0.50 g were removed from individual mice, homogenized in phosphate-buffered saline, and centrifuged at 2000 rpm for 10 min (at 4 °C). Malondialdehyde (MDA) measurements from supernatants were obtained using the lipid peroxidation assay kit (Sigma-Aldrich, USA). Briefly, 500 µL of supernatant was pipetted into a new microtube and 2.5 mL of trichloroacetic acid5% solution was subsequently added before being centrifuged at 2000 rpm for 10 min at 4 °C. Next, 1.5 mL of supernatant was transferred into a new microtube and 1.5 mL of thiobarbituric acid 1% solution was added followed by a brief vortex and subsequent incubation in the water bath for 30 min at 100 °C. Next, the sample was put at room temperature for 15 min followed by the measurement of the absorbance using a spectrophotometer (SmartSpecTM Plus Spectrophotometer, BioRad Laboratories, USA) at an optical density of 530 nm. The catalase activity was determined using a catalase activity assay kit (Colorimetric/Fluorometric, Abcam-ab83464, UK) as per the manufacturer's instructions. Briefly, the supernatant of tissue homogenate was incubated at 25 °C for 5 min then 12 µL of 1 mM H_2O_2 solution was added and the sample was incubated at 25 °C for 30 min. Thereafter, the 10 µL stop solution was added followed by the 50 µL developer mix before being incubated at 25 °C for 10 min. Eventually, the absorbance

was measured using a microplate reader at an OD 570 nm (xMark 1681150, Bio-Rad Laboratory Inc., USA).

Measurement of interleukin-6 mRNA expression in WAT

WAT samples (0.3 g) were collected and immediately fixed in RNAlater™ solution (Sigma-Aldrich, USA) before being stored at -80 °C. Total RNA was subsequently isolated using an RNA isolation kit (RNeasy Mini Kit Cat. #74104, Qiagen, Germany) and RNA concentration was measured using a NanoDrop-1000 (OD 260/280; Thermo Fisher Scientific, USA). cDNA synthesis of interleukin-6 (IL-6) was achieved *via* reverse transcriptase polymerase chain reaction (PCR) using the Tetro™ cDNA synthesis kit (Meridian Bioscience, USA). The mRNA expression of IL-6 in the WAT was measured by real-time quantitative PCR (qRT-PCR; using iQ SYBR Green Supermix; Bio-Rad Laboratories, USA). The temperature and time conditions of the machine were set as follows: 95 °C for 2 min then 95 °C for 5 s then 63.5 °C for 30 s (repeated for 40 cycles). The temperatures for the melting curve were 65 to 95°C with an increment of 0.5 °C every 5 s. Primers sets (respectively indicated as forward and reverse primers) for IL-6: 5'-CTGCAAGAGACTTCCATCCAG-3' and 5'-AGTGGTATAGACAGGTCTGTTGG-3' and β-actin: 5'-GGCCAACCGTGAAAAGATGA-3' and 5'-CAGCCTGGATGGCTACGTACA-3' were used during qRT-PCR amplification. The primer sequences were determined based on the previous study (16). The specificity of the primers for the IL-6 gene of mice (*Mus musculus* L.) was confirmed by primer blast analysis (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Cycle threshold (CT) values were recorded and the relative expression level of IL-6 mRNA was calculated and expressed as a fold change against β-actin (17).

Gut microbiota analysis via 16S ribosomal RNA gene sequencing

Fecal matter in the caeca was freshly sampled and weighed (at 50 mg) for individual mice. To prevent contamination, gloves and sterilized instruments were used during processing. Fecal samples were immediately frozen at -80 °C. Genomic DNA of fecal microbes was extracted using the DNeasy Powersoil Pro kit (Qiagen) according to the manufacturer's protocols. Thereafter, PCR amplification of the hypervariable V3-V4 regions of the bacterial 16S rRNA gene was achieved using 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') primers (12). Purified PCR products were submitted to Novogene (Singapore) for sequencing using the Illumina Novaseq 6000 platform. Obtained NGS data were analyzed using the 16S Metagenomics GAIA ver. 2.0 software (http://www.metagenomics.cloud, Sequentia Biotech, Spain).

Data analysis

The data were presented as mean values ± SEM, with the difference among groups being determined using ANOVA followed by the Bonferroni post hoc test. In addition, for the data on body weight, the paired t-test was also deployed. P values < 0.05 were considered significant.

RESULTS

Effect of YBF on body weight and adipose tissues

After four weeks of treatment, HFD-fed mice's body weights significantly differed compared to that of mice fed with HFD + 10% YBF (Fig. 1A). In contrast, the body weights of HFD + 10% YBF mice (but not HFD + YBF 2.5% mice) were statistically comparable with that of the ND group ($P > 0.05$). The result of paired samples t-test showed that, as compared with their respective initial values (before the treatment), the body weight of the HFD-fed group was significantly increased earlier (in two weeks of treatment $P < 0.01$) followed by the HFD + YBF 2.5% group (in four weeks of treatment; $P < 0.01$) then HFD + YBF 10% group (in six weeks of treatment; $P < 0.01$). Body weight gain had been statistically significant for mice fed with HFD as compared to HFD + 2.5 % YBF ($P < 0.05$) and HFD + 10% YBF ($P < 0.01$) as well as the ND treatment ($P < 0.01$) (Fig. 1B). Determination of eWAT and iBAT masses were used to

confirm the prevention of adiposity in HFD mice that were provided with YBF supplements. While statistically comparable with the ND group ($P > 0.05$), HFD + 10% YBF mice had significantly lower eWAT masses than those of the HFD group ($P < 0.01$; Fig. 1C). Yet, the eWAT masses of HFD + 2.5% YBF mice were statistically comparable to the HFD group ($P > 0.05$). In terms of iBAT mass, the HFD-fed mice showed lower masses ($P < 0.05$) compared to all other treatments (HFD + 2.5% YBF mice, HFD + 10% YBF mice, and ND mice) (Fig. 1D).

Microscopic observations of eWAT samples indicated that mice fed with HFD + 2.5% YBF and HFD + 10% YBF had smaller white adipocytes than mice fed with HFD only ($P < 0.05$) (Fig. 2A and B). Although ND group adipocyte sizes were statistically similar to that of HFD + 10% YBF mice ($P > 0.05$), they were significantly different from that of HFD + 2.5% YBF mice ($P < 0.05$). Meanwhile, mice in the HFD + 10% YBF-fed group had smaller brown adipocytes compared to that of HFD-fed mice and HFD + 2.5% YBF-fed mice ($P < 0.01$; Fig. 2C and D). The size of adipocytes was found to be similar between the HFD + 10% YBF and ND groups ($P > 0.05$).

To examine the counteractive effect of YBF on oxidative stress and inflammation in WAT, the MDA level, catalase activity, and mRNA expression of IL-6 were determined. The MDA levels within the WAT of HFD + 2.5% YBF and HFD + 10% YBF groups were significantly lower than that of the HFD-fed group ($P < 0.05$; Fig. 2E**)**. However, no significant difference could be detected between the ND-fed and HFD + YBF-fed mice ($P > 0.05$).

The HFD + 2.5% YBF and HFD + 10% YBF mice showed significantly higher ($P < 0.05$) catalase activity than the HFD-fed mice (Fig. 2F). mRNA expression of IL-6 in WAT had been profoundly lower in HFD + 10% YBF-fed mice compared to HFD-fed and HFD + 2.5% YBF mice ($P < 0.01$; Fig. 2G). In contrast, IL-6 mRNA expression was similar in HFD + 10% YBF and ND mice ($P > 0.05$).

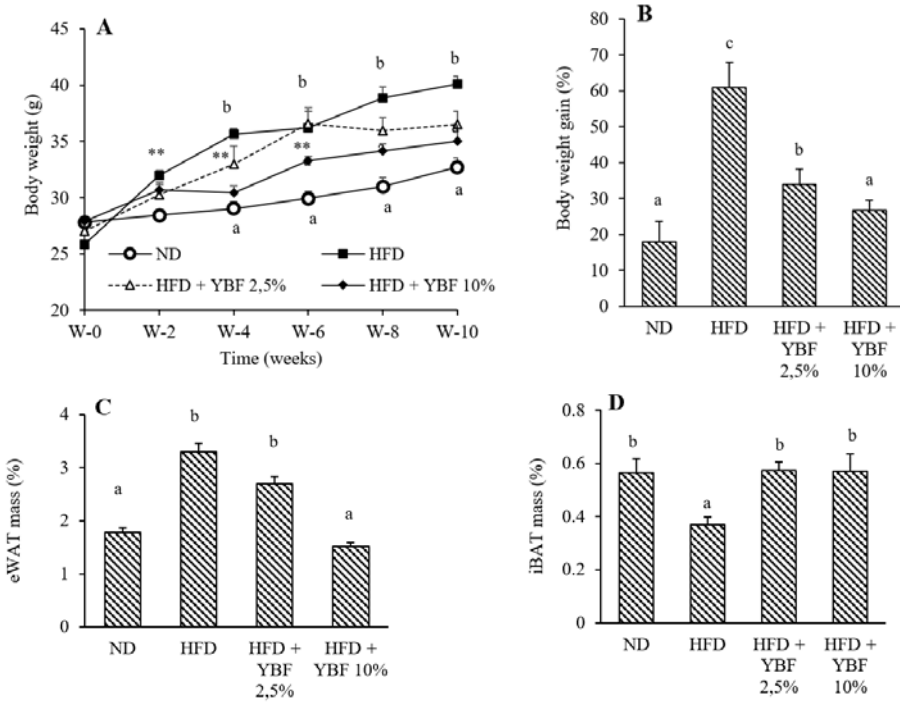

Fig. 1. Effect of YBF on mice body weight and adipose tissue mass. (A) Body weight as measured every two weeks; (B) percentage of body weight gain after ten weeks of treatment; (C) the eWAT mass; and (D) the iBAT mass presented against total body weight. Data are presented as mean ± SEM. The different lowercase letters above the bars indicate statistical differences ($P < 0.05$); $^{**}P < 0.01$ indicates a significant difference between initial body weight (before treatments) and respective body weight in the same group based on paired samples t-test. YBF, Yam bean fiber; ND, normal diet; HFD, high-fat diet; eWAT, epididymal white adipose tissue; iBAT, interscapular brown adipose tissue.

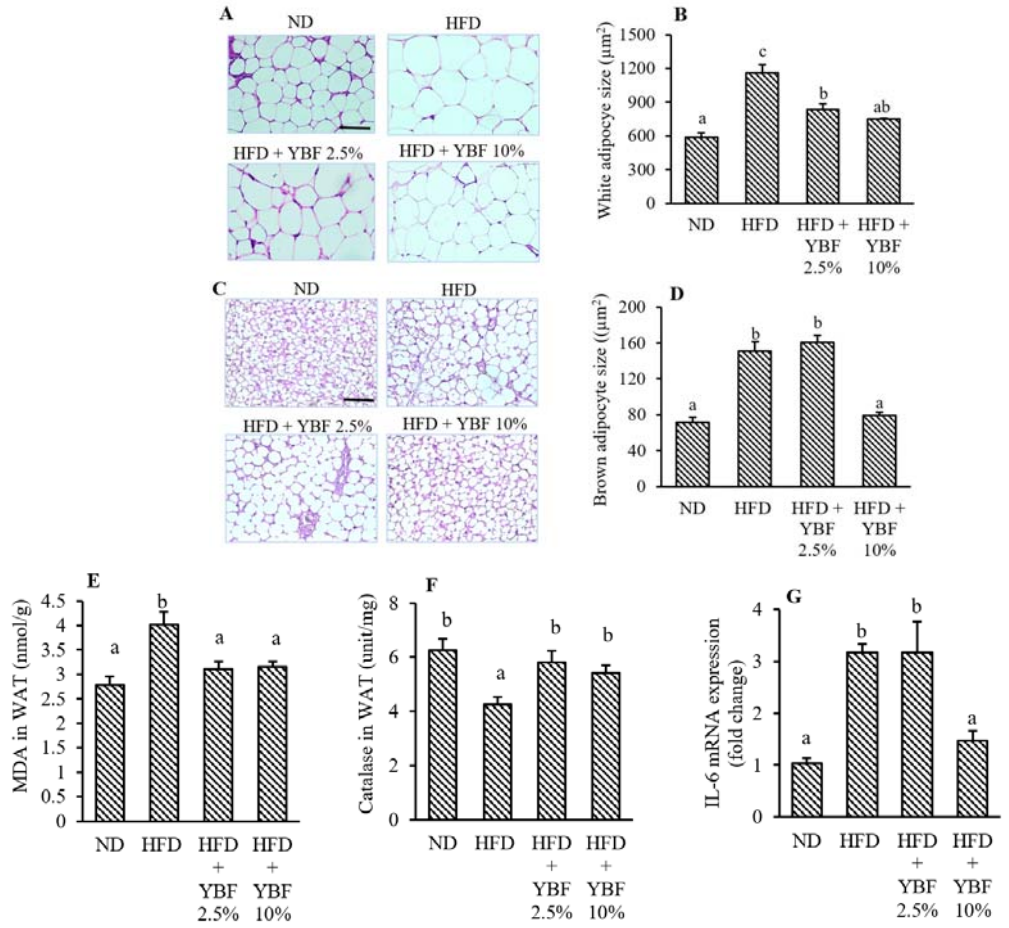

Fig. 2. Effect of YBF on microscopic structure, oxidative stress, and inflammation in adipose tissue. (A) Histology of eWAT depicting white adipocytes; (B) mean of white adipocyte size; (C) histology of iBAT depicting brown adipocytes; (D) mean of white adipocyte size; (E) MDA level in WAT; (F) catalase activity in WAT; (G) mRNA expression of IL-6 in WAT. The tissues in A and C were stained with hematoxylin-eosin. Scale bars in A and C: 20 μm. Data are presented as mean ± SEM. The different lowercase letters above the bars indicate statistical differences ($P < 0.05$);. YBF, Yam bean fiber; ND, normal diet; HFD, high-fat diet; eWAT, epididymal white adipose tissue; iBAT, interscapular brown adipose tissue; MDA, malondialdehyde; IL-6, interleukin-6.

Effect of YBF on blood glucose and insulin levels

At the beginning of the experiment, all groups of mice presented with normal blood glucose levels (77.33-96.17 mg/dL; Fig. 3A). Thereafter, a significant increase ($P < 0.05$) in blood glucose levels had been observed for HFD-fed mice (150.33 ± 10.84 mg/dL). A similar result was observed for fasting blood glucose levels with HFD + 2.5% YBF and HFD + 10% YBF mice showing significantly lower blood glucose levels than HFD-fed mice ($P < 0.05$; Fig. 3B).

Plasma insulin levels were significantly lower in the HFD + 10% YBF and ND mice compared to that of HFD-fed mice ($P < 0.05$; Fig. 3C). As indicated by their lower blood sugar levels at 15, 30, 60, and 90 min after the i.p. injection, HFD + 2.5% YBF and HFD + 10% YBF mice had better glucose tolerance than HFD mice (Fig. 3D). Moreover, the AUC values of glucose tolerance showed significant reductions for HFD + 2.5% YBF and HFD + 10% YBF mice, compared to HFD-fed mice ($P < 0.05$; Fig. 3E). Based on a marked reduction in blood glucose after the i.p. insulin injection, HFD + 2.5% YBF and HFD + 10% YBF mice had higher insulin sensitivities than HFD mice (Fig. 3F). The AUC values of insulin tolerance were also significantly lower in the HFD + 2.5% YBF and HFD + 10% YBF mice compared to HFD-fed mice ($P < 0.01$; Fig. 3G).

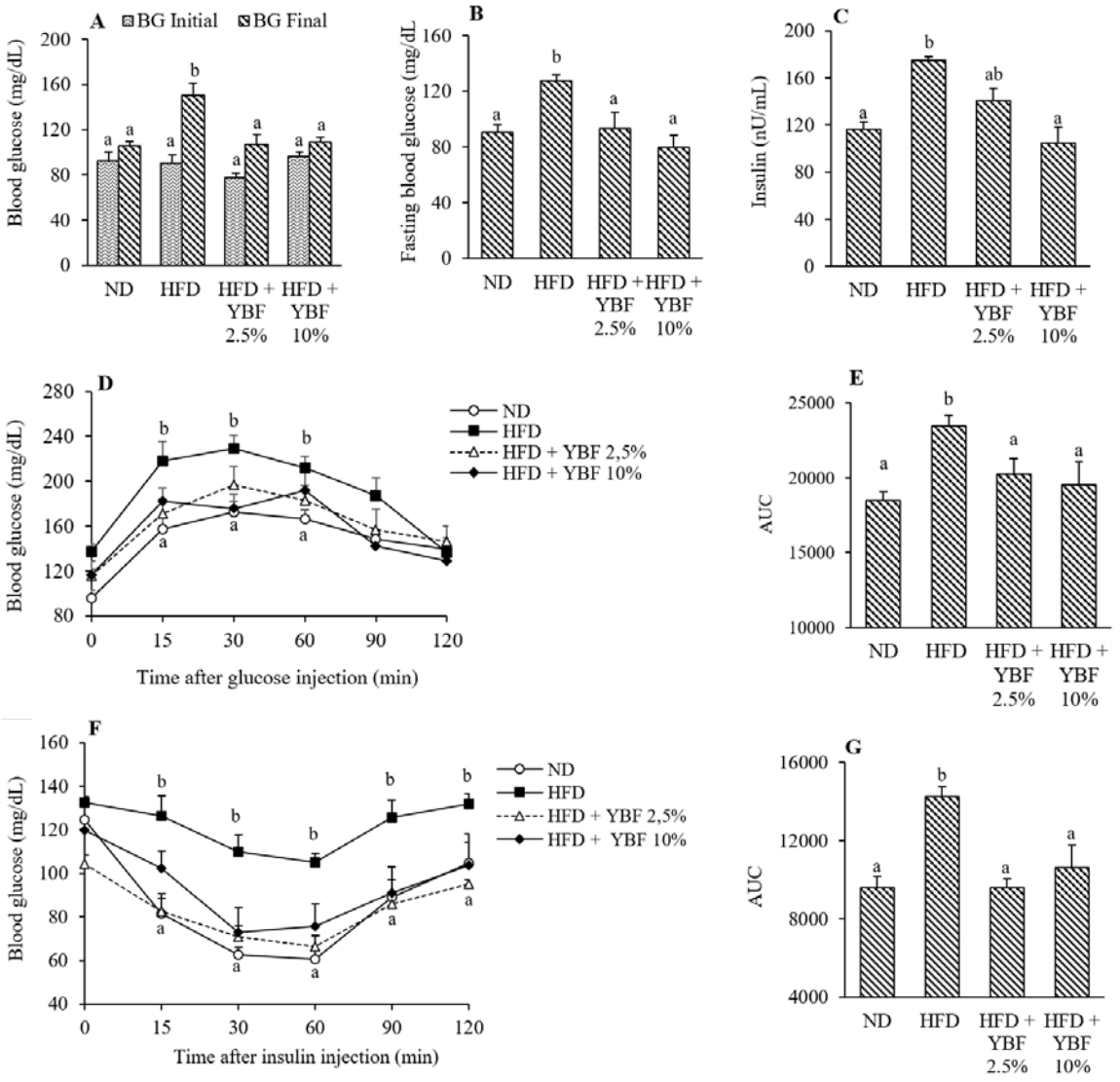

Fig. 3. Effect of YBF on blood glucose and insulin. (A) Blood glucose levels before and after treatment; (B) fasting blood glucose; (C) plasma insulin level; (D) blood glucose levels in i.p. GTT; (E) area under the curve calculated based on the i.p. GTT; (F) blood glucose in the i.p. ITT, (G) AUC calculated based on ITT. Data are presented as mean ± SEM. The different lowercase letters above the bars indicate statistical differences ($P < 0.05$). YBF, Yam bean fiber; ND, normal diet; HFD; high-fat diet; AUC, area under the curve; GTT, glucose tolerance test; ITT, insulin tolerance test.

Effect of YBF on gut microbiota composition and colonic health

In this study, the composition of gut microbiota was investigated *via* 16S rRNA sequencing. The phylum-level composition results indicated a dominance of Firmicutes and Bacteroidota in all treatment groups (Fig. 4A). Compared to HFD-fed mice (Bacteroidota: 0.23%; Firmicutes: 0.55%; Desulfobacterota: 0.16%), HFD + 10% YBF-fed mice showed increased Bacteroidota (0.46%) and reduced Firmicutes (0.46%) and Desulfobacterota (0.01%) representation. In contrast, HFD + 2.5% YBF-fed mice showed higher and lower abundances of Firmicutes/Desulfobacterota and Bacteroidota, respectively (compared to that of HFD + 10% YBF-fed mice). The ND-fed mice also had a marked dominance of Firmicutes (0.79%) over Bacteroidota (0.16%) alongside a low abundance of Desulfobacterota.

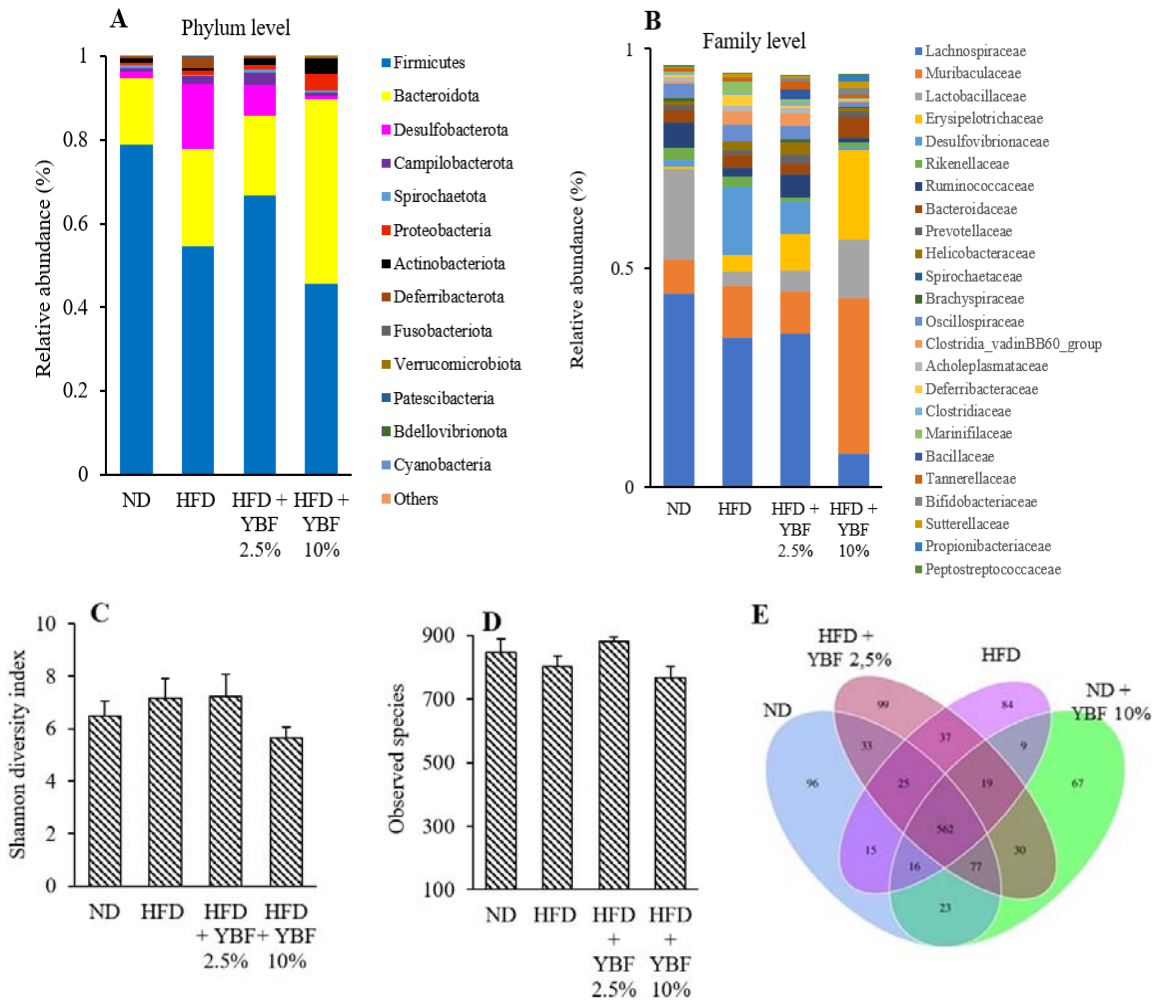

Fig. 4. Effect of YBF on gut microbiota composition and diversity. (A) Composition of gut microbiota presented in relative abundance at the phylum level; (B) relative abundance at family level; (C) Shannon-Weiner diversity index; (D) number of observed species; (E) Venn diagram depicting species similarity among groups of treatment. YBF, Yam bean fiber; ND, normal diet; HFD, high-fat diet.

The family-level microbial analysis (Fig. 4B) highlighted five common families namely Lachnospiraceae (Firmicutes), Muribaculaceae (Bacteroidota), Lactobacillaceae (Firmicutes), Erysipelotrichaceae (Firmicutes), and Desulfovibrionaceae (Desulfobacterota) that exhibited dynamic alterations in abundance across all treatment groups. For example, HFD + 10% YBF mice showed a marked reduction in Lachnospiraceae and Desulfovibrionaceae (4.6 and 19.2 times lower than that of HFD mice) and a notable increase in Muribaculaceae, Lactobacillaceae, and Erysipelotrichaceae (3.0, 4.3, and 5.4 times higher than that of HFD mice). The gut microbiota composition of HFD + 2.5% YBF mice resembled that of HFD mice, although the former had slightly higher and lower abundances of Erysipelotrichaceae and Desulfovibrionaceae, respectively. The ND group showed a noticeable increase in Lactobacillaceae, while the HFD group exhibited an increase in Desulfovibrionaceae. The HFD + 10% YBF group had a marked dominance of Muribaculaceae and Erysipelotrichaceae. Statistical analyses on species diversity (i.e. Shannon diversity indices) revealed that HFD (7.16) and HFD + 2.5% YBF (7.24) mice had slightly higher gut microbiota diversity indices than ND (6.49) and HFD + 10% YBF (5.64) mice (Fig. 4C-D).

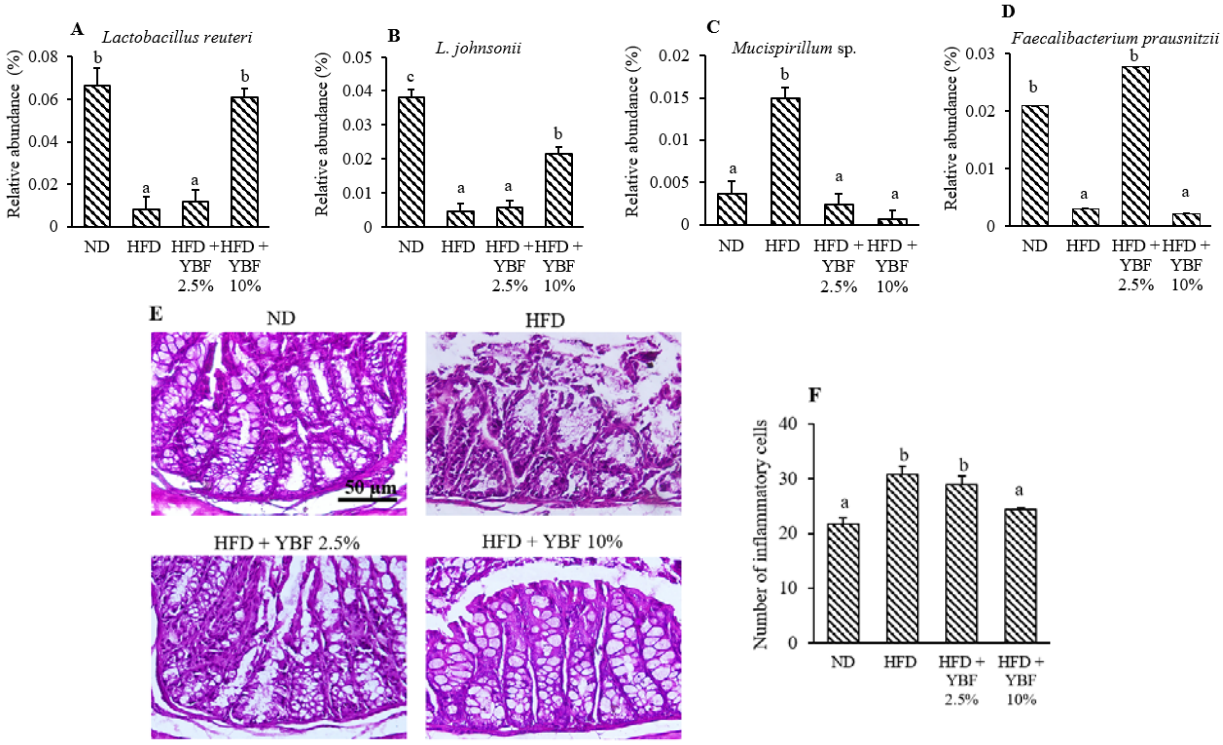

Fig. 5. Effect of YBF on gut microbiota species and colon histopathology. (A-D) Relative abundance of common microbiota species; (E) histology of colon stained by hematoxylin and eosin; (F) mean of the colon inflammatory cell number. Data are presented as mean ± SEM. The different lowercase letters above the bars indicate statistical differences ($P < 0.05$). YBF, Yam bean fiber; ND; normal diet, HFD; high-fat diet;

The species number was found to be highest for HFD + 2.5% YBF mice (882 species), followed by ND mice (847 species), HFD mice (803 species), and HFD + 10% YBF mice (767 species). While 562 similar microbial species were found across all treatment groups (Fig. 4E), the uniqueness of species had been highest in the HFD + 2.5% YBF group (99 unique species), followed by the ND group (96 unique species), HFD + 10% YBF group (84 unique species), and the HFD group (67 unique species). The HFD + 10% YBF and HFD + 2.5% YBF mice, respectively exhibited the least (606) and highest (688) species similarities to the HFD group.

Four species namely *Lactobacillus reuteri* (Firmicutes, Lactobacillaceae), *L. johnsonii* (Firmicutes, Lactobacilaceae), *Mucispirillum* sp. (Deferribacterota, Defferibacteriaceae), and *Faecalibacterium prausnitzii* (Firmicutes, Ruminococcaceae) exhibited profound alterations in their abundance across all treatment groups. Specifically, *L. reuteri* and *L. johnsonii* had been highly abundant in the ND and HFD + 10% YBF groups and less abundant in the HFD and HFD + 2.5% YBF groups (Fig. 5A and B). Meanwhile, *Mucispirillum* sp. had been noticeably abundant in the HFD-fed group and less abundant in the other groups (Fig. 5C), while *F. prausnitzii* had been abundant in the ND and HFD + 2.5% YBF groups but less abundant in the HFD and HFD + 10% YBF groups (Fig. 5D).

Histopathological examinations illustrated profound structural damages in the colon tissues of HFD-fed mice whereas no damage could be observed for the other groups (Fig. 5E). HFD-fed mice colons showed mucosal thinning and thereby reduced villi and crypt depths as well as erosion and epithelial layer desquamation of the lamina propria and submucosa edema. These histopathological alterations were less notable in the HFD + 2.5% YBF and HFD + YBF 10% groups. Lastly, the number of inflammatory cells in the colon had been significantly lower for HFD + 10% YBF mice compared to HFD and HFD + 2.5% YBF mice ($P < 0.05$; Fig. 5F).

DISCUSSION

Findings from the present study demonstrated the effectiveness of extracted fiber from YBF in counteracting some indicators of HFD-induced metabolic diseases including adiposity, hyperglycaemia, and insulin resistance. Moreover, YBF enhanced the abundance of some health-promoting gut microbiota and precluded histopathological alterations of the colon.

According to previous studies in both animals and humans, excessive intake of an HFD can cause rapid fat deposition in visceral adipose tissues and subsequently increased body weight which, in turn, can lead to the development of obesity (18-20). Yet, sufficient intake of dietary fiber could contribute to a lower prevalence of obesity (21). In this study, a higher dosage of YBF (10%) prevented the HFD-induced increase of visceral WAT mass and body weight in mice while also increasing iBAT mass (thus suggesting a counteractive effect). Moreover, it was found that YBF precluded the HFD-induced hypertrophy of white and brown adipocytes. This was in line with a previous study, where bamboo shoot fiber (10%) was effective in reducing body weight, WAT mass, and white adipocyte size in HFD-fed mice (12).

Although a lower dose of YBF (2.5%) under the HFD challenge exerted a significant effect on 10-week body weight gain and iBAT mass, this dosage was unable to reduce WAT mass and hypertrophy of white and brown adipocytes. As such, a higher dose of YBF is required to effectively counteract HFD-induced adiposity.

Previous studies have indicated that overnutrition-induced hypertrophy of adipocytes could lead to hypoxia in adipose tissues (22,23). Inadequate O_2 supply has also been closely associated with the elevation of oxidative stress, cell death, and the overexpression of pro-inflammatory factors (24,25). In this study, the hypertrophic WAT of HFD-fed mice exhibited a substantial elevation of MDA (a marker of oxidative stress) and depletion of catalase activity (an endogenous antioxidant) while overexpressing proinflammatory factor IL-6. The supplementation of a higher dose of YBF indicated a counteractive effect against HFD by significantly decreasing MDA and IL-6 expression while increasing catalase activity in WAT. This was in line with findings from a previous murine experiment where psyllium dietary fiber was effective in inhibiting oxidant-induced MDA production (26). Another study, using mice and human hybridoma cell line-HB4C5, indicated that YBF could exert an immunomodulatory effect (9). The prevention of oxidative stress and inflammation of WAT in the present study might thus be due to adipocyte hypertrophy and hypoxia aversion. This speculation is considered based on the fact that in the 10% YBF-treated group, the smaller size of white adipocytes (non-hypertrophic and non-hypoxic cells) exhibited lower oxidative stress and inflammation markers. Furthermore, these effects may be associated with the direct action of YBF on the immune system.

Short-chain fatty acids (SCFAs), the products of fiber fermentation by gut microbiota, can also contribute to the prevention of oxidative stress and inflammation in WAT. It has been reported that SCFAs (particularly propionate and butyrate) could effectively exert anti-inflammatory and antioxidant effects (27,28). There are two possible suggested mechanisms underlying the higher dose of YBF in decreasing the MDA and IL-6 and increasing the catalase activity in WAT under HFD treatment. Firstly, as described previously (29), SCFAs could inhibit oxidative stress while promoting endogenous antioxidant activity *via* activation of the nuclear erythroid 2-related factor 2 (Nrf2) signaling pathway in the cells. Therefore, an increase in SCFAs production due to high-fiber intake could lead to the prevention of MDA accumulation (oxidative stress byproduct) while sustaining high catalase activity (an endogenous antioxidant) within the WAT. Secondly, SCFAs are also capable of sustaining the balance between anti- and proinflammatory cytokines by acting *via* free acid receptors in the immune cells (30). Accordingly, an increase in SCFAs production due to high dietary fiber intake might effectively prevent the expression of proinflammatory cytokines including IL-6 within the WAT. However, since SCFA levels

were not determined for YBF treatments in the present study, it remains unclear whether YBF incorporation increased SCFA production and subsequently mitigated HFD-induced inflammation and oxidative stress.

It is known that detrimental effects of a fatty diet such as local hypoxia, oxidative stress, and inflammation could lead to insulin resistance (thereby developing T2DM) (31,32). In this study, HFD-fed mice exhibited insulin resistance and increased fasting blood glucose levels as well as impaired sugar tolerance and hyperinsulinemia. However, supplementation with a higher dose of YBF effectively prevented such effects. This finding is in line with the previous report demonstrating that YBF (at the dose of 10 and 25%) effectively prevented elevated blood glucose levels, islet hypertrophy, and ectopic adiposity in the pancreas of HFD-fed mice (33). While the specific route for YBF to preclude HFD-induced insulin resistance remains to be elucidated, causes for insulin resistance have been observed through various routes (e.g. disruption of the signaling system, dysregulation of GLUT4 synthesis, and alterations of adipokines) (23).

One suggested a mechanism by which dietary fiber may exert beneficial effects on health is through the modulation of gut microbiota composition and diversity (34,35). Findings from the present study indicated that a higher dosage of YBF altered the microbiota composition at the phylum level. Specifically, a reduction in Firmicutes and Desulfobacterota dominance over Bacteroidota within HFD-fed mice had been observed in the YBF group. An increase in Bacteroidota has previously been associated with better health indices in HFD-fed mice when supplemented with dietary fiber from konjac glucomannan (34). Another study with human subjects suggested an inhibitory effect of dietary fiber against Desulfobacterota (which had been decreased by agave inulin (36), while a cohort study showed increased Desulfobacterota prevalence in T2DM patients (37). Taken together, this suggests a close association between Desulfobacterota and metabolic dysregulation.

Species-level analyses showed that a higher dosage of YBF enhanced the abundance of health-benefit microbes (i.e. *L. reuteri* and *L. johnsonii*) while reducing the abundance of pathogenic microbes (*Mucispirillum* sp.). This suggests that species abundance modulation might be a plausible mechanism employed by YBF in preventing obesity, insulin resistance, inflammation, and histopathological alterations of the colon. These results were supported by previous findings that *L. reuteri* could inhibit pathogenic microbes and positively restore the composition of commensal gut microbiota (38,39). A study on piglets also indicated that *L. johnsonii* increased intestinal immunity (40), while oral administration of this microbe in HFD-fed mice significantly decreased body weight gain, WAT mass, and ameliorated insulin sensitivity (41).

Conversely, an increment of *Mucispirillum* spp. was found to be strongly associated with the prevalence of inflammatory bowel disease (42) and an increase in body weight (43). Interestingly, in the present study, both lower and higher doses of YBF decreased *Mucispirillum* sp. abundance, although the lower YBF dose failed to increase the abundance of *L. reuteri* and *L. johnsonii* under the HFD challenge. However, this dosage promoted the prevalence of *F. prausnitzii* (a species reported to protect the intestine against colitis and inflammation) (44). It might, therefore, be speculated that bioactive substances and physical properties of YBF contributed to the dose-dependent response of gut microbiota composition and diversity when incorporated in HFD. Subsequent phytochemical and physical characterizations of YBF should thus be explored in further studies.

The composition of the YBF (particularly the proportion of soluble and insoluble fiber) might determine the dynamics of the gut microbial community. Fibers, particularly soluble fiber is a highly suitable fermentative substrate for common health-promoting gut microbiota thus allowing them to utilize it as energy resources and expand their populations (45). As a result, the domination of such species of microbiota in the gut might outperform the other species that are unable to ferment the fiber. In addition, the bioactive compounds that might be incorporated in the YBF could also

possibly affect the microbial activity and growth in the gut thereby modulating their composition. It has been described that the yam bean tuber contains various phytochemicals including triterpenoids, flavonoids, organic acids, and fatty acids (5) that may exert modulatory effects on gut microbiota composition. Subsequent phytochemical and physical characterizations of YBF should thus be explored in further studies.

In our study, a lower dose of YBF (2.5%) was not effective in mitigating the detrimental effects of HFD, particularly adiposity and inflammation both in WAT and colon. We suggest that a lower dose of YBF may have a limited level of the water-soluble fiber as a fermentative substrate for health-promoting microbiota in the gut thus limiting the production and the subsequent physiological outcomes of SCFAs. As a result, gut dysbiosis, adiposity, and inflammation remain observed in mice treated with a lower dose of YBF.

The present study had several limitations. Firstly, only two dose levels of YBF (2.5% and 10%) were tested which prevented further determinations regarding the most effective dosage in precluding detrimental effects of HFD. Secondly, our current study did not clarify the possible detrimental health effect of a high dose of YBF (10%). A previous study found that incorporation of the YBF at the dose of 25%, but not 10%, significantly reduced water intake in mice fed with a high-sucrose diet (10) which may lead to dehydration and constipation. However, further investigation is absolutely required to determine the other possible detrimental effects of excessive consumption of YBF. Thirdly, expression levels of anti-inflammatory cytokines were not measured. As such, the association(s) of the inflammatory response was unclear (i.e. reduction induced by YBF *vs* the stimulation of anti-inflammatory factors). Moreover, the study did not elucidate whether YBF affected other metabolic hormones (e.g. glucagon-like peptide 1, leptin and ghrelin).

CONCLUSION

Results from the present study showed that dietary fiber extracted from a 10% dosage of YBF could effectively counteract HFD-induced adiposity, hyperglycaemia, and insulin resistance. In addition to significantly reducing oxidative stress and inflammation, YBF further increased endogenous antioxidant activity in WAT. Moreover, YBF increased the abundance of health-promoting microbial species, while inhibiting a pathogenic species and precluded HFD-induced histopathological alterations and inflammation of the colon. The YBF diet supplements have been proven to be useful in preventing diet-induced metabolic diseases, gut microbiota dysbiosis, and related health problems. Our present findings in mice as the animal model should be followed by future studies concerning the safety of YBF as a supplemental diet and its therapeutic effects against other diet-induced diseases (i.e. non-alcoholic fatty liver disease, cardiovascular disease). Moreover, clinical investigations involving human subjects with certain metabolic disorders (including obesity and type 2 diabetes mellitus) are also required to be done.

Acknowledgments

This work was financially supported by the Ministry of Education, Culture, Research and Technology of Indonesia through World Class Research Grant (WCR No. 011/E5/PG.02.00.T/2022; the Fiscal Year 2022).

Conflict of interest statement

All authors declare there is no conflict of interest in this study.

Authors' contributions

P. Santoso, R. Maliza, R. Rahayu contributed to the conceptualization of the study, experimental design, data analysis, and manuscript preparation; P. Santoso, R. Maliza, Y. Astrina, F. Syukri, and S. Maharani performed the experiments and measurements; R. Rahayu was responsible for project administration. The final version of the article was approved by all authors.

REFERENCES

1. Yang XF, Qiu YQ, Wang L, Gao KG, Jiang ZY. A high-fat diet increases body fat mass and up-regulates expression of genes related to adipogenesis and inflammation in a genetically lean pig. J Zhejiang Univ Sci B. 2018;19(11):884-894. DOI: 10.1631/jzus.B1700507.

2. Gao M, Ma Y, Liu D. High-fat diet-induced adiposity, adipose inflammation, hepatic steatosis and hyperinsulinemia in outbred CD-1 mice. PLoS One. 2015;10(3):e0119784,1-15. DOI: 10.1371/journal.pone.0119784.
3. Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: are they linked? Gut Microbes. 2017;8(2):130-142. DOI: 10.1080/19490976.2016.1270811.
4. Saklayen MG. The global epidemic of the metabolic syndrome. Curr Hypertens Rep. 2018;20(2):12,1-8. DOI: 10.1007/s11906-018-0812-z.
5. Aiswal V, Chauhan S, Lee H-J. The bioactivity and phytochemicals of *Pachyrhizus erosus* (L.). urb: a multifunctional underutilized crop plant. Antioxidants. 2022;11(1):58,1-23. DOI: 10.3390/antiox11010058.
6. Park CJ, Han J-S. Hypoglycemic effect of jicama (*Pachyrhizus erosus*) extract on streptozotocin-induced diabetic mice. Prev Nutr Food Sci. 2015;20(2):88-93. DOI: 10.3746/pnf.2015.20.2.88.
7. Park CJ, Lee H-A, Han JS. Jicama (*Pachyrhizus erosus*) extract increases insulin sensitivity and regulates hepatic glucose in C57BL/Ksj-db/db mice. J Clin Biochem Nutr. 2016;58(1):56-63. DOI: 10.3164/jcbn.15-59.
8. Thaptimthong T, Kasemsuk T, Sibmooh N, Unchern S. Platelet inhibitory effects of juices from *Pachyrhizus erosus* L. root and *Psidium guajava* L. fruit: a randomized controlled trial in healthy volunteers. BMC Complement Altern Med. 2016;16:269,1-12. DOI: 10.1186/s12906-016-1255-1.
9. Kumalasari ID, Nishi K, Harmayani E, Raharjo S, Sugahara T. Immunomodulatory activity of bengkoang *(Pachyrhizus erosus*) fiber extract *in vitro* and *in vivo.* Cytotechnology. 2014;66(1):75-85. DOI: 10.1007/s10616-013-9539-5.
10. Santoso P, Amelia A, Rahayu R. Jicama (*Pachyrhizus erosus*) fiber prevents excessive blood glucose and body weight increase without affecting food intake in mice fed with high-sugar diet. J Adv Vet Anim Res. 2019;6(2):222-230. DOI: 10.5455/javar.2019.f336.
11. Wang ZQ, Yu Y, Zhang XH, Floyd ZE, Boudreau A, Lian K*, et al.* Comparing the effects of nano-sized sugarcane fiber with cellulose and psyllium on hepatic cellular signaling in mice. Int J Nanomedicine. 2012;7:2999-3012. DOI: 10.2147/IJN.S30887.
12. Li X, Guo J, Ji K, Zhang P. Bamboo shoot fiber prevents obesity in mice by modulating the gut microbiota. Sci Rep. 2016;6:32953,1-11. DOI: 10.1038/srep32953.
13. Sunarti, Rini SLS, Sinorita H, Ariani D. Effect of fiber-rich snacks on C-reactive protein and atherogenic index in type 2 diabetes patients. Rom J Diabet Nutr Metab Dis. 2018;25(4):357-362. DOI:rjdnmd.org/index.php/RJDNMD/article/view/531.
14. Ding S, Jiang J, Yu P, Zhang G, Zhang G, Liu X. Green tea polyphenol treatment attenuates atherosclerosis in high-fat diet-fed apolipoprotein E-knockout mice *via* alleviating dyslipidemia and up-regulating autophagy. PLoS One. 2017;12(8):e0181666,1-18. DOI: 10.1371/journal.pone.0181666.
15. Yao L, Wei J, Shi S, Guo K, Wang X, Wang Q*, et al*. Modified lingguizhugan decoction incorporated with dietary restriction and exercise ameliorates hyperglycemia, hyperlipidemia and hypertension in a rat model of the metabolic syndrome. BMC Complement Altern Med. 2017;17(1):132,1-12. DOI: 10.1186/s12906-017-1557-y.
16. Keinicke H, Sun G, Mentzel CMJ, Fredholm M, John LM, Andersen B, *et al*. FGF21 regulates hepatic metabolic pathways to improve steatosis and inflammation. Endocr Connect. 2020;9(8):755-768. DOI: 10.1530/EC-20-0152.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods.2001;25(4):402-408. DOI: 10.1006/meth.2001.1262.
18. Li J, Wu H, Liu Y, Yang L. High fat diet induced obesity model using four strains of mice: kunming, C57BL/6, BALB/c and ICR. Exp Anim. 2020;69(3):326-335. DOI: 10.1538/expanim.19-0148.
19. Wang B, Kong Q, Li X, Zhao J, Zhang H, Chen W, *et al.* A high-fat diet increases gut microbiota biodiversity and energy expenditure due to nutrient difference. Nutrients. 2020;12(10):3197,1-20. DOI: 10.3390/nu12103197.
20. Feng R, Sun G, Zhang Y, Sun Q, Ju L, Sun C, *et al*. Short-term high-fat diet exacerbates insulin resistance and glycolipid metabolism disorders in young obese men with hyperlipidemia, as determined by metabolomics analysis using ultra-HPLC-quadrupole time-of-flight mass spectrometry. J Diabetes. 2019;11(2):148-160. DOI: 10.1111/1753-0407.12828.
21. Ruhee RT, Suzuki K. Dietary fiber and its effect on obesity, Adv Med Res. 2018;1:1,1-14. DOI: 10.12715/amr.2018.1.2
22. Engin A. Adipose tissue hypoxia in obesity and its impact on preadipocytes and macrophages: hypoxia hypothesis. Adv Exp Med Biol.2017;960:305-326. DOI: 10.1007/978-3-319-48382-5_13.
23. Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev. 2013;93(1):1-21. DOI:10.1152/physrev.00017.2012.
24. Revelo XS, Luck H, Winer S, Winer DA. Morphological and inflammatory changes in visceral adipose tissue during obesity. Endocr Pathol. 2014;25(1):93-101. DOI: 10.1007/s12022-013-9288-1.
25. Netzer N, Gatterer H, Faulhaber M, Burtscher M, Pramsohler S, Pesta D. Hypoxia, oxidative stress and fat. Biomolecules. 2015;5(2):1143-1150. DOI: 10.3390/biom5021143.
26. Ogata M, Van Hung T, Tari H, Arakawa T, Suzuki T. Dietary psyllium fiber increases intestinal heat shock protein 25 expression in mice. Nutr Res. 2017;39:25-33. DOI: 10.1016/j.nutres.2017.02.002.

27. Segain JP, de la Bletiere DR, Bourreille A, Leray V, Gervois N, Rosales C *et al*. Butyrate inhibits inflammatory responses through NF kappa B inhibition: implications for Crohn's disease. Gut. 2000;47(3):397-403. DOI: 10.1136/gut.47.3.397.
28. Filippone A, Lanza M, Campolo M, Casili G, Paterniti I, Cuzzocrea S, *et al*. The anti-inflammatory and antioxidant effects of sodium propionate. Int J Mol Sci. 2020; 21(8):3026,1-17. DOI: 10.3390/ijms21083026.
29. González-Bosch C, Boorman E, Zunszain PA, Mann GE. Short-chain fatty acids as modulators of redox signaling in health and disease. Redox Biol. 2021;47:102165,1-11. DOI:10.1016/j.redox.2021.102165.
30. Ratajczak W, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczyńska M. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). Acta Biochim Pol. 2019;66(1):1-12. DOI:10.18388/abp.2018_2648.
31. Rasouli N. Adipose tissue hypoxia and insulin resistance. J Investig Med. 2016;64(4):830-832. DOI: 10.1136/jim-2016-000106.
32. Lawler HM, Underkofler CM, Kern PA, Erickson C, Bredbeck B, Rasouli N. Adipose tissue hypoxia, inflammation, and fibrosis in obese insulin-sensitive and obese insulin-resistant subjects. J Clin Endocrinol Metab. 2016;101(4):1422-1428. DOI: 10.1210/jc.2015-4125.
33. Santoso P, Maliza R, Insani SJ, Fadhila Q, Rahayu R. Preventive effect of jicama (*Pachyrhizus erosus)* fiber against diabetes development in mice fed with high-fat diet. J Appl Pharm Sci. 2021;11(1):137-143. DOI: 10.7324/JAPS.2021.110116.
34. Zhai X, Lin D, Zhao Y, Li W, Yang X. Effects of dietary fiber supplementation on fatty acid metabolism and intestinal microbiota diversity in C57BL/6J mice fed with a high-fat diet. J Agric Food Chem. 2018;66(48):12706-12718. DOI: 10.1021/acs.jafc.8b05036.
35. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe. 2018;23(6):705-715. DOI: 10.1016/j.chom.2018.05.012.
36. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey Jr GC, Swanson KS. Agave inulin supplementation affects the fecal microbiota of healthy adults participating in a randomized, double-blind, placebo-controlled, crossover trial. J Nutr. 2015;145(9):2025-2032. DOI: 10.3945/jn.115.217331.
37. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490(7418):55-60. DOI: 10.1038/nature11450.
38. Abhisingha M, Dumnil J, Pitaksutheepong C. Selection of potential probiotic *Lactobacillus* with inhibitory activity against *Salmonella* and fecal coliform bacteria. Probiotics Antimicrob Proteins. 2018;10(2):218-227. DOI: 10.1007/s12602-017-9304-8.
39. Wang P, Li Y, Xiao H, Shi Y, Le G-W, Sun J. Isolation of *Lactobacillus reuteri* from Peyer's patches and their effects on sIgA production and gut microbiota diversity. Mol Nutr Food Res. 2016;60(9):2020-2030. DOI: 10.1002/mnfr.201501065.
40. Xin J, Zeng D, Wang H, Sun N, Zhao Y, Dan Y, *et al*. Probiotic *Lactobacillus johnsonii* BS15 promotes growth performance, intestinal immunity, and gut microbiota in piglets. Prob Antimicrob Prot. 2020;12(1):184-193. DOI: 10.1007/s12602-018-9511-y.
41. Yang G, Hong E, Oh S, Kim E. Non-viable *Lactobacillus johnsonii* JNU3402 protects against diet-induced obesity. Foods. 2020;9(10):1494,1-11. DOI: 10.3390/foods9101494.
42. Herp S, Durai Raj AC, Salvado Silva M, Woelfel S, Stecher B. The human symbiont *Mucispirillum schaedleri*: causality in health and disease. Med Microbiol Immunol. 2021; 210(4):173-179. DOI: 10.1007/s00430-021-00702-9.
43. Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, *et al*. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and netabolic syndrome. Cell Metab. 2015;22(3):516-530. DOI: 10.1016/j.cmet.2015.07.007.
44. Miquel S, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, *et al*. *Faecalibacterium prausnitzii* and human intestinal health. Curr Opin Microbiol. 2013;16(3):255-261. DOI:10.1016/j.mib.2013.06.003.
45. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the gastrointestinal microbiome with nondigestible fermentable carbohydrates to improve human health. Microbiol Spectr. 2017;5(5):1-24. DOI: 10.1128/microbiolspec.BAD-0019-2017.