Supplementation of yam bean (*Pachyrhizus erosus* L.) fiber ameliorates dyslipidemia, liver pathology and hypersecretion of metabolic hormones in mice fed a high-fat diet

Putra Santoso*[ID], Rita Maliza[ID]

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

A B S T R A C T

Introduction: Yam bean (*Pachyrhizus erosus* L.) offers numerous health benefits. However, the effects of its dietary fiber (yam bean fiber, YBF) on dyslipidemia, liver disease, and the overproduction of metabolic hormones, specifically glucagon-like peptide-1 (GLP-1) and fibroblast growth factor 21 (FGF21), resulting from a high-fat diet (HFD) remain underexplored. Thus, our present investigation sought to address this gap.
Methods: Adult male mice (n = 24) were randomly assigned into four different groups such as normal diet (ND) as a control group, HFD, and HFD supplemented with either 2.5% or 10% YBF. After a 12-week dietary regimen, plasma lipid profiles, liver histology and biochemistry, and the levels of FGF21 and GLP-1 were assessed.
Results: YBF supplementation, especially at 10% dose, effectively lowered total serum cholesterol, triglyceride (TG), and low-density lipoprotein (LDL) compared with those fed HFD ($P < 0.05$). YBF also reduced liver weight and mitigated the elevation of malondialdehyde (MDA) and the depletion of catalase (CAT) activity induced by HFD in liver tissues ($P < 0.05$). Furthermore, 10% YBF supplementation effectively countered liver pathology, including central vein enlargement, hepatic steatosis, inflammation, abnormal sinusoids, and hepatocyte degeneration caused by HFD ($P < 0.05$). YBF at 10% also attenuated the HFD-induced hypersecretion of FGF21 and GLP-1 hormones.
Conclusion: Our findings indicate that YBF supplementation could counteract the adverse effects of HFD, particularly in terms of dyslipidemia, liver disease, and metabolic hormone imbalances. Incorporating YBF into diets may thus offer protective benefits against HFD-induced metabolic diseases and associated health issues.

Implication for health policy/practice/research/medical education:
This research revealed the therapeutic potential of YBF against health issues from HFD, such as dyslipidemia, liver damage, and hormonal imbalances. Incorporating YBF in diets may be useful to preclude these HFD-related diseases.

Introduction

Yam bean tuber (*Pachyrhizus erosus* L., Leguminosae) is commonly consumed in Latin America, Asia, and Africa as fresh or processed food. The yam bean tuber highly contains various compounds (1), which have been demonstrated to offer numerous health benefits, including antidiabetic (2), cardioprotective (3), antioxidant (4), and gastroprotective properties (5). Every 100 g of fresh yam bean tuber contains approximately 1.4-6% of dietary fiber (1). Several bioactive compounds, including inulin, pectin, daidzein, genistein, and pterocarpan, have been identified in the yam bean tuber (1,4,6). In many countries, yam bean tubers are harvested to extract starch while the by-product of extraction is considered as wastes (6). In fact, the by-product is rich in dietary fiber (yam bean fiber, YBF) eliciting various medicinal effects against diseases. A prior report demonstrated that YBF exerts an immunomodulatory effect in mice (7). Furthermore,

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*Corresponding author: Putra Santoso,
Email: putrasantoso@sci.unand.ac.id

YBF has a protective effect against diabetes mellitus caused by diet containing excessive sucrose (8). Another study revealed that YBF can counteract the pathological alterations in the liver of mice fed with a high-sucrose drink (9). However, the effects of YBF in countering the deleterious outcomes caused by high-fat diet (HFD), including dyslipidemia, hepatic steatosis, and dysregulated metabolic hormones, remained unknown.

HFD has been strongly linked to the development of diverse health problems such as including diabetes, adiposity, inflammation, and cardiovascular disease (10). Moreover, HFD is a major cause of dyslipidemia, hepatic steatosis, and dysregulated metabolic hormones (11). On the other hand, metabolic hormones, namely glucagon-like peptide-1 (GLP-1) and fibroblast growth factor 21 (FGF21), have been suggested to play potent synergistic and additive roles in maintaining metabolic homeostasis (12,13). Administration of a dual agonist for GLP-1/FGF21 has been shown to effectively alleviate obesity, type 2 diabetes, and hepatic steatosis in animal models (14). However, excessive consumption of HFD leads to the hypersecretion of both GLP-1 (15) and FGF21 hormones (16).

Proper consumption of dietary fiber has been suggested to counteract metabolic dysregulation. For instance, a study in mice demonstrated that dietary fiber from bamboo shoots is capable of improving blood glucose and plasma lipid profiles, while modulating microbiota composition in the intestine of HFD-fed mice (17). Moreover, an investigation in people with metabolic syndrome found that dietary fiber intake derived from maize could improve insulin sensitivity in adipose and muscle tissues (18). Dietary fiber can also combat HFD-induced dyslipidemia by binding to bile acids, preventing their reabsorption (19). This compels the liver to use more cholesterol for bile acid production, thereby lowering blood cholesterol. Soluble fiber is also capable of forming a gel in the gut, which traps fats and reduces their absorption (20). Fiber intake further promotes production of short-chain fatty acid (SCFA) through intestinal microbiota fermentation thereby inhibiting cholesterol synthesis reshapes liver fat metabolism to reduce triglyceride and cholesterol synthesis (17). However, different sources of fiber may exert varying degrees of effectiveness in counteracting diseases. For example, fibers derived from various plants used as salad vegetables exhibit different efficacies in inhibiting carbohydrate digestion and glucose absorption in vitro (19). This variation might be attributed to the differences in composition (soluble and non-soluble fiber), phytochemical constituents, and physicochemical characteristics of the fiber (20). As such, it is speculated that YBF might also have varying degrees of effectiveness in mitigating the detrimental effects of HFD. Unfortunately, up to now, studies investigating the medicinal benefits of YBF, particularly in counteracting HFD-induced dyslipidemia, hepatic steatosis, and hypersecretion of metabolic hormones, are limited. Revealing the counteractive effects of YBF on dyslipidemia, hepatic steatosis, and hypersecretion of metabolic hormones caused by HFD might provide valuable insights into the prevention and treatment of these conditions. Given the high prevalence of these health problems worldwide (10,13), finding effective and safe interventions is of utmost importance. YBF may be a promising candidate for natural-based remedies, and its potential health benefits against metabolic disorders warrant advanced investigation.

Given that scientific evidence regarding the medicinal benefits of YBF in combating liver disease and dysregulated metabolic hormones caused by HFD remain limited, this current report is the first in defining the counteractive effects of YBF against dyslipidemia, hepatic steatosis, and hypersecretion of FGF21 and GLP-1 hormones induced by HFD. The study aimed to determine whether supplementing the diet with YBF could effectively mitigate dyslipidemia, liver disease, and the dysregulation of metabolic hormones against HFD.

Materials and Methods

Collection of jicama tuber and fiber extraction
Yam bean tubers were freshly harvested from the farm in Padang Pariaman, West Sumatra. The species identity of the plant was validated by a certified botanist in the Herbarium of Andalas University (Specimen No: 62/ANDA-1/022). After collection, the yam bean tubers were cleaned and peeled before being sliced into small pieces, and then ground to achieve a porridge-like consistency. Fiber extraction from the sample followed a protocol described previously (21). In brief, the sample was placed in a jar filled with distilled water (1 part sample to 4 parts distilled water) and stored at 4 °C overnight. Subsequently, the fiber fraction was separated as a supernatant on the top layer inside the jar. This fiber was then carefully collected, filtered, and steamed for 30 minutes. Following this, it was dried in an electric oven for 17 hours at 67-68°C. After that, the dried sample was pulverized and the fiber powder was kept in an isolated container at a room temperature.

Experimental treatment on animal models
This study involved 24 adult male mice (2 months old; weighing 25-27 g; DDY strain) obtained from the Baso Veterinary Center (Bukittinggi, West Sumatra). Before the experiment, the mice were acclimated for 7 days in the designated room with a sustained temperature (25-26 °C), humidity (66-67%), and illumination (12-hour light and 12- hour dark periods). The animals were housed in an individual cage (one mouse per cage) and fed ad libitum with a commercial rodent diet (Citra Ina Fedmill, Jakarta, Indonesia) and tap water. Subsequently, the animals were

randomly divided into four equal groups based on their respective diets:

- Group 1: normal diet (ND)
- Group 2: high-fat diet (HFD)
- Group 3: HFD supplemented with 2.5% YBF
- Group 4: HFD supplemented with 10% YBF

The dietary experiments were carried out for 12 weeks. The diet composition and the doses of YBF were determined based on a previous study (21). The detailed diet compositions are presented in Table 1. The fat was from milkfat (Fernleaf, Malaysia), carbohydrate was from refined corn starch (Bogasari, Indonesia), protein was from whey protein (Vectorlab, Indonesia), and the vitamin and mineral mix was procured from BioServ (USA). The ND was a commercial diet containing 4% fat, which equates to a 10.47% energy contribution to the total energy of the diet according to the manufacturer's information (Citra Ina Fedmill, Jakarta). The Institutional Research Ethics Board of Andalas University evaluated and approved the experimental procedures of the study (No. 528/KEP.UA/022).

Measurements of plasma lipids

The plasma lipid profiles were examined at the end of the treatments using a method as previously described (11). The blood was drawn from the urethane-euthanized mice through cardiac puncture and subjected to centrifugation for 10 minutes at 3000 RPM. Subsequent to this, the plasma lipids namely triglyceride (TG), low-density lipoprotein (LDL), total cholesterol (TC) and high-density lipoprotein (HDL) were determined using colorimetric lipid quantification kits from Cell Biolabs (San Diego, USA).

Measurements of body weight gain and blood glucose

Body weight gain was determined by measuring body weight at the beginning and end of the treatment using a digital scale (ACIS-AW, Shanghai). Blood glucose was also measured at the beginning and end of the treatment using a glucometer (Accu-Chek, Farmaku) by sampling the blood drop from the tail vein.

Observation of liver morphology, weight, and histopathology

At the final day of the treatment, the animals were euthanized using a lethal dose of ketamine (via i.p injection). The liver organs were then gently removed, weighed, and photographed for gross morphological observations before being fixed for 12 hours in formalin solution (neutral buffered formalin, NBF; Sigma Aldrich). Following this, samples were processed according to protocols for tissue slide preparation described elsewhere (22). The tissues were stained with hematoxylin-eosin. Finally, tissue slides (5 representative tissue slices with 5 view fields for each mouse) were observed under a microscope (Olympus, Tokyo, Japan). Measurements of the central vein diameter, fat vesicle count, inflammatory cell count, degenerated cell count, and abnormal sinusoids were conducted based on the photomicrographs using ImageJ software for Windows (The National Institute of Health, USA).

Measurements of malondialdehyde (MDA) and catalase (CAT) in the liver

MDA and CAT measurements were conducted according to procedures previously described (21). At the end of treatment, 0.3 g of liver tissues were chopped and then homogenized in phosphate-buffered saline. The homogenates were subsequently centrifuged for 10 minutes at 2000 RPM to obtain the supernatants. MDA levels were measured using an assay kit (Lipid peroxidation assay; Cell Biolabs, USA), following the manufacturer's protocols. The SmartSpec™ Plus Spectrophotometer (Bio-Rad Lab, USA; OD 530 nm) was deployed to measure the absorbance of samples. CAT levels were assessed using a colorimetric/fluorometric catalase assay kit (Abcam-ab83464, UK) as per protocol provided by the manufacturer. An xMark 1681150 microplate reader (Bio-Rad Laboratory Inc., USA; OD 570 nm) was used to determine the absorbance of the samples.

Determination of plasma GLP-1 and FGF21 levels

The procedures for measuring GLP-1 and FGF21 hormones were based on the methods described in previous studies (23,24). At the end of the experiment, blood was drawn and the plasma was obtained through the centrifugation (temperature at 4 °C, speed at 3000 RPM for 10 minutes). The samples were kept at -80 °C until needed. The FGF21 and GLP-1 levels were determined using the mouse FGF21

Table 1. Diet compositions used in the experiment

Materials	Proportion (%)			
	ND	HFD	HFD + 2.5% YBF	HFD + 10% YBF
Protein	20	20	20	20
Fat	4	25	25	25
Carbohydrate	59.3	42.3	39.8	32.3
YBF	0	0	2.5	10
Cellulose	4	0	0	0
Mineral and vitamin mix	12.7	12.7	12.7	12.7

Note: ND, normal diet;, HFD, high-fat diet; YBF, Yam bean fiber.

and GLP-1 enzyme-linked immunosorbent assay kits Bioassay Tech., Shanghai Lab, China), respectively. The sample absorbances were determined using a microplate reader (x-Mark-1681150 Bio-Rad, USA).

Statistical analysis

Quantitative data are depicted as mean ± SE. Levene statistic test was deployed to determine the data homogeneity and the Shapiro-Wilk test was used to assessed data normality. Subsequent analysis of variance was conducted, followed by the Bonferroni Post Hoc test, to determine statistically significant differences of the data. $P < 0.05$ was set as significant level. The statistical analyses were conducted using IBM SPSS version 25.

Results

Effects of YBF on plasma lipid profiles

Plasma lipid profiles were evaluated after 12 weeks of treatment. The results revealed that HFD significantly elevated TC (Figure 1A), LDL (Figure 1B), and TG (Figure 1C) in mice when compared to ND ($P < 0.05$). In contrast, supplementation with YBF at doses of 2.5% and 10% substantially reduced TC and TG levels ($P < 0.05$). However, only the higher dose of YBF (10%) was able to significantly lower LDL levels ($P < 0.05$), while the lower dose of YBF (2.5%) had a similar effect as HFD alone on LDL levels ($P > 0.05$). Additionally, HDL levels were not significantly different among all groups of the experiment ($P > 0.05$) (Figure 1D).

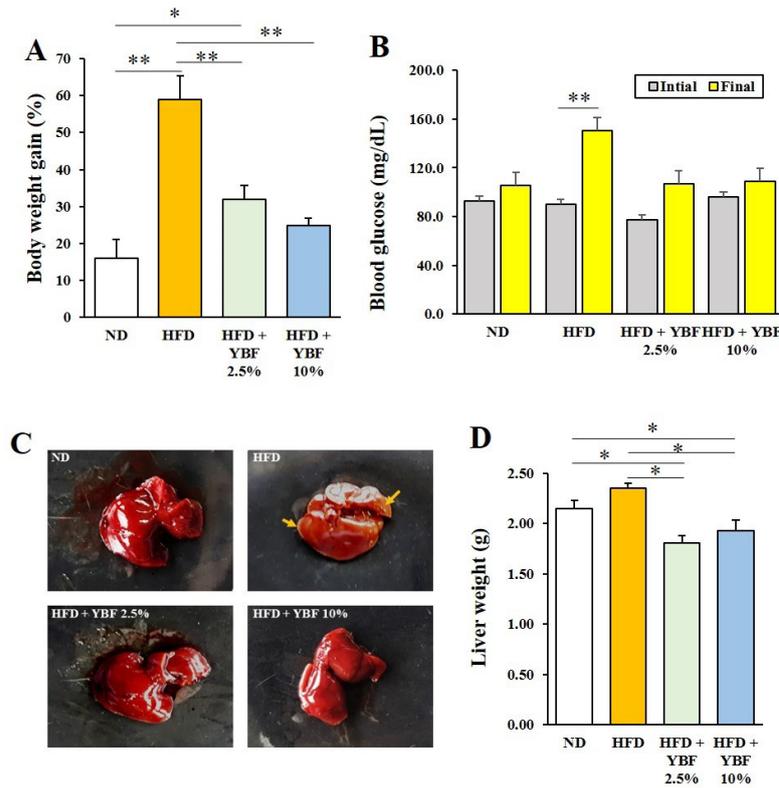### Effects of YBF on body weight, blood glucose and liver pathology

The mice fed with HFD exhibited more significant increase in body weight gain than those fed with ND ($P < 0.01$) (Figure 2A). However, those fed with HFD supplemented with YBF at doses of 2.5% and 10% experienced significantly less weight gain ($P < 0.05$ and $P < 0.01$, respectively) than the HFD group. Additionally, blood glucose levels (Figure 2B) in the HFD group substantially elevated at the final day of treatment ($P < 0.05$). In contrast, all other groups maintained lower blood glucose levels.

Gross morphological examination (Figure 2C) revealed that the livers of HFD-fed mice displayed a yellowish color, indicative of visible fat accumulation. In comparison, the livers of mice from other groups (ND and YBF-fed) appeared dark red and lacked visible fat deposits. Furthermore, measurements of liver weight (Figure 2D) showed that the HFD-fed group had the heaviest livers, though the weight was statistically similar to the ND-fed mice ($P > 0.05$). Unlikely, the mice fed with YBF (2.5% and 10%) had significantly lighter livers than both HFD and ND groups ($P < 0.05$).

The mice fed with HFD exhibited significantly higher MDA levels than the ND group ($P < 0.05$). Conversely, those fed with YBF at doses of 2.5% and 10% displayed
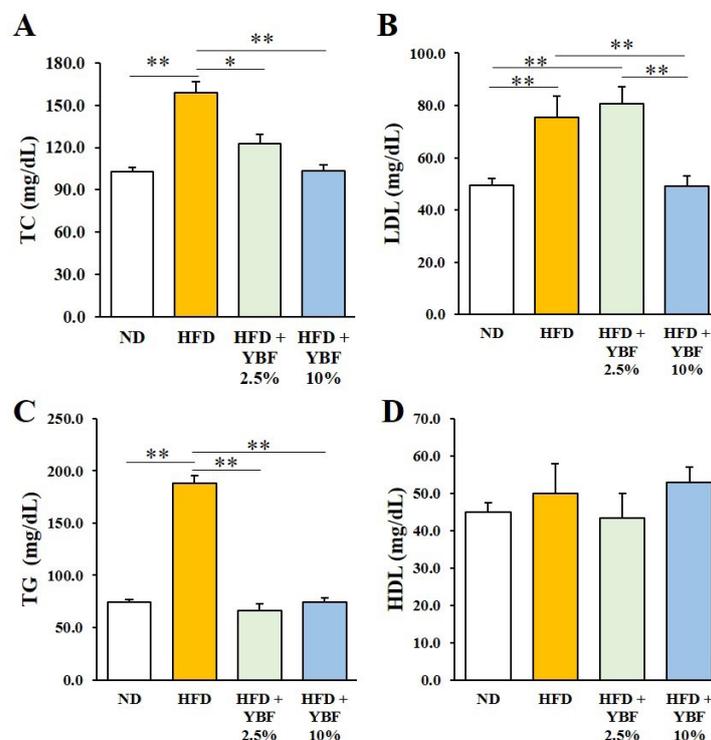

Figure 1. Effects of yam bean fiber on plasma lipid profiles in mice. (A) level of plasma cholesterol (TC), (B) level of low-density lipoprotein (LDL), (C) level of triglyceride (TG), (D) level of high-density lipoprotein (HDL). (*) $P < 0.05$; (**) $P < 0.01$ based on the Bonferroni post hoc test; ND (normal diet); HFD (high-fat diet); YBF (yam bean fiber).

Figure 2. Effects of yam bean fiber on body weight gain, blood glucose level and liver morphology and weight. (A) body weight gain at the end of treatment, (B) blood glucose at the beginning and end of treatment, (C) gross morphology of liver, (D) liver weight measured at the end of treatment. (*) $P < 0.05$; (**) $P < 0.01$ based on the Bonferroni post hoc test; ND (normal diet); HFD (high-fat diet); YBF (yam bean fiber); yellow arrows in C indicate fat accumulation in the liver of HFD-fed mice.

substantially lower MDA levels than the HFD group ($P < 0.05$) but were statistically similar to the ND group ($P > 0.05$). Furthermore, CAT activity levels in the liver (Figure 3B) were markedly lower in the HFD-fed group than ND group ($P < 0.05$). Otherwise, the mice fed with YBF at doses of 2.5% and 10% exhibited significantly higher CAT activity levels than the HFD group ($P < 0.05$). CAT activity levels in the YBF-fed groups remained lower than ND group ($P < 0.05$).

A histopathological examination on the liver tissues (Figure 4) revealed that HFD caused highly dilated central veins. Further measurements of the central vein size (Figure 5A) indicated that HFD-fed mice had a significantly wider central vein diameter than the ND group ($P < 0.01$). Notably, the mice fed with 10% YBF, but not 2.5% YBF, had a significantly smaller central vein diameter, though it still differed significantly from the ND group ($P < 0.05$). Additionally, HFD-induced
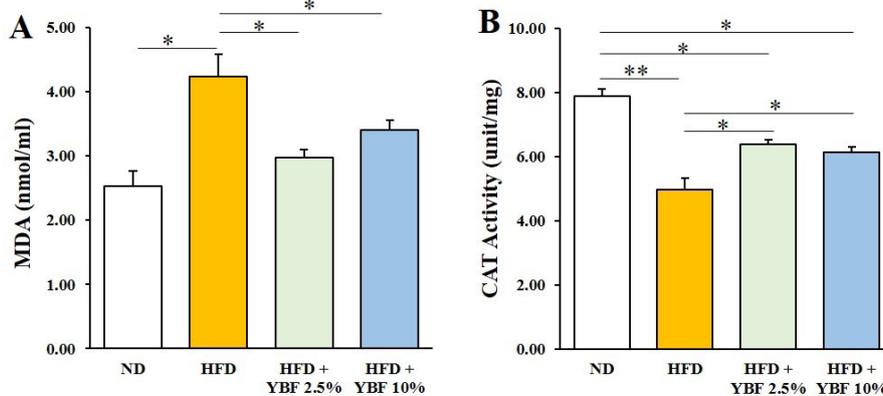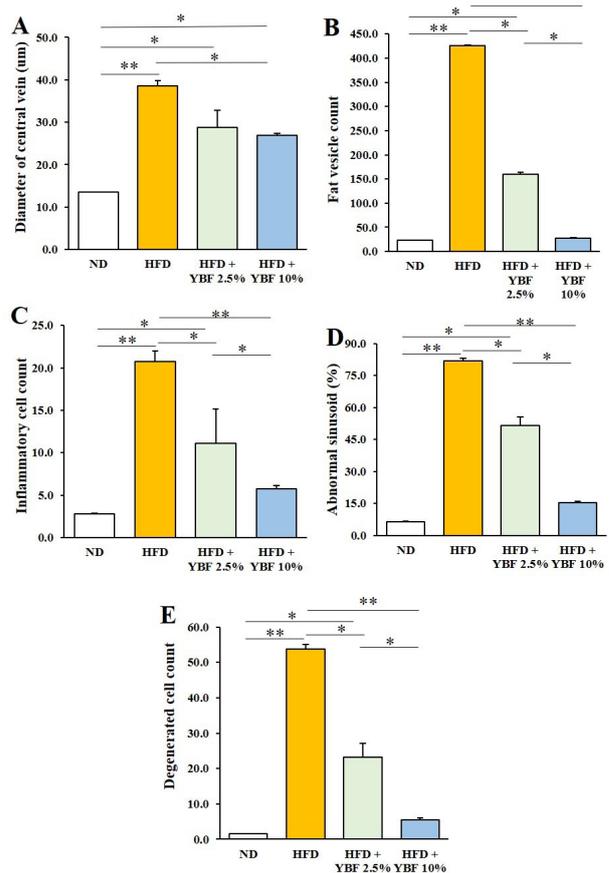

Figure 3. Effect of yam bean fiber on oxidative stress in the liver. (A) Malondialdehyde (MDA) levels in the liver tissue, (B) catalase activity (CAT) levels in liver tissue. (*) $P < 0.05$; (**) $P < 0.01$ based on the Bonferroni post hoc test; ND (normal diet); HFD (high-fat diet); YBF (yam bean fiber).
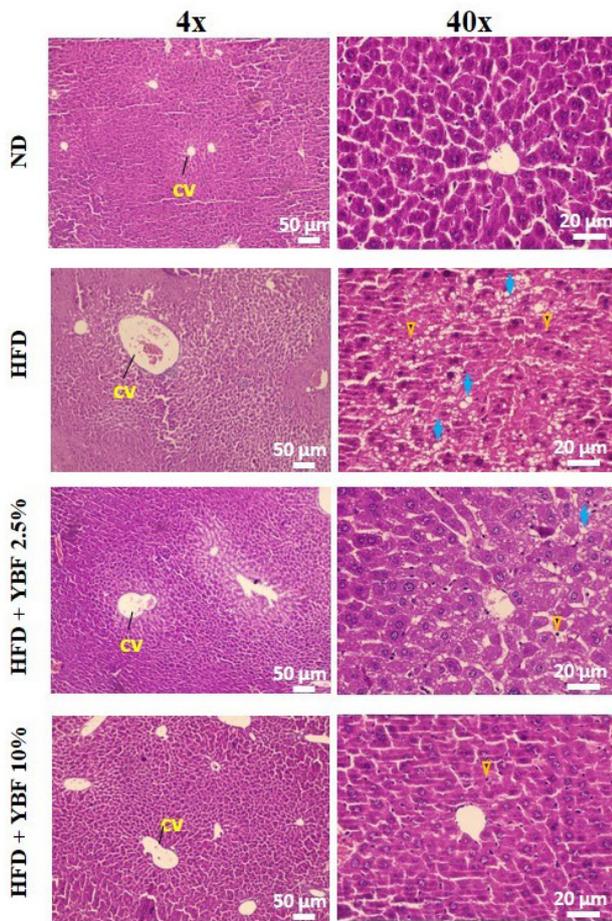
Figure 4. Representative photomicrographs of mice liver. 4x (lower magnification pictures), 40x (higher magnification pictures), blue arrows in right panels indicate fat vesicle, yellow arrows indicate inflammatory cells. CV (central vein); ND (normal diet); HFD (high-fat diet); YBF (yam bean fiber).
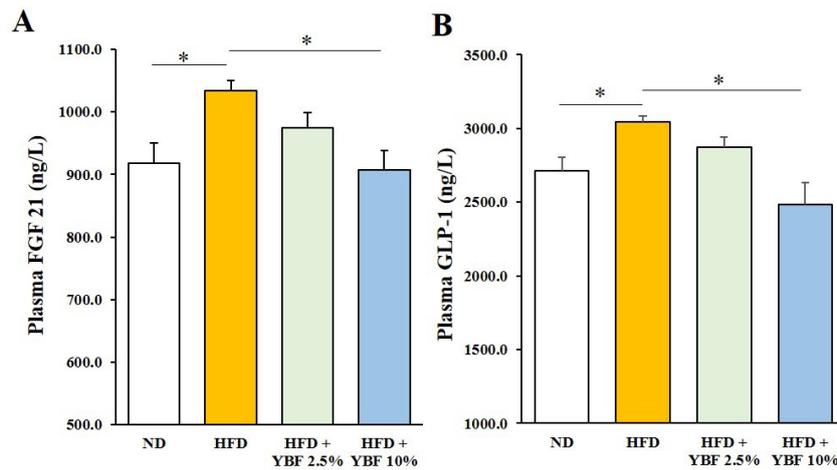

Figure 5. Effects of yam bean fiber on the liver histopathology of mice. (A) average central vein diameter, (B) average number of fat vesicle per view field, (C) average number of inflammatory cells per view field, (D) percentage of abnormal sinusoid per field view, (E) average of degenerated hepatocyte per view field. (*) $P < 0.05$; (**) $P < 0.01$ based on the Bonferroni post hoc test; ND (normal diet); HFD (high-fat diet); YBF (yam bean fiber).

excessive hepatic steatosis, evident from the abundance of intracellular fat vesicles (Figure 4). Counting these hepatic fat vesicles, the indicators of hepatic steatosis (Figure 5B) showed a pronounced increase in the HFD-fed mice compared to the ND group ($P < 0.01$). Conversely, YBF-fed mice exhibited fewer fat vesicles in their livers than the HFD group ($P < 0.05$). Notably, the number of fat vesicles in the 10% YBF-fed group was statistically similar to the ND-fed group ($P > 0.05$). The distribution of macrophages, indicative of inflammation, was also pronounced in the livers of HFD-fed mice. The counts of these macrophages or inflammatory cells (Figure 5C) revealed a significant increase in the HFD-fed mice compared to the ND group ($P < 0.01$). However, YBF supplementation significantly reduced the number of macrophages compared to the HFD group ($P < 0.05$), and at the 10% dosage, YBF lowered the macrophage count to levels comparable with the ND group ($P > 0.05$). Additionally, HFD led to the enlargement of liver sinusoids and wider sinusoid pits. The percentage of abnormal sinusoids (Figure 5D) was considerably higher in HFD-fed mice than in ND-

fed mice ($P < 0.01$). In contrast, YBF-fed mice showed fewer abnormal sinusoids than the HFD-fed group ($P < 0.05$), and at the 10% dosage, YBF resulted in sinusoid abnormalities comparable to the ND-fed group ($P > 0.05$). Moreover, HFD induced hepatocyte degeneration, evident from noticeable cytoplasmic vacuoles and necrosis. The count of these degenerated hepatocytes (Figure 5E) was substantially elevated in mice fed with HFD as compared with those fed with ND ($P < 0.01$). YBF incorporation in the diet reduced the number of degenerated cells significantly when compared to the HFD alone ($P < 0.05$ and $P < 0.01$ for 2.5% and 10% YBF, respectively). At the 10% dosage, YBF decreased the number of degenerated hepatocytes to levels similar to the ND group ($P > 0.05$).

Effects of YBF on FGF21 and GLP-1 hormones
Measurements of plasma FGF21 and GLP-1 levels (Figure 6) were conducted after 12 weeks of diet treatments. As depicted in Figure 6A, HFD led to an apparent rise in FGF21 levels ($P < 0.05$). Conversely, the mice fed with 10% YBF, but not 2.5% YBF, exhibited significantly lower

Figure 6. Effect of yam bean fiber on the level of metabolic hormones. (A) levels of plasma FGF21, (B) levels of plasma GLP-1 in mice. (*) $P < 0.05$ based on the Bonferroni post hoc test; ND (normal diet); HFD (high-fat diet); YBF (yam bean fiber).

plasma FGF21 levels than the HFD group ($P < 0.05$). Furthermore, the FGF21 levels in the 10% YBF-fed group were comparable to those in the ND-fed group ($P > 0.05$). Moving to the GLP-1 levels (Figure 6B), it was observed that the HFD-fed group had significantly higher GLP-1 levels compared to the ND group ($P < 0.05$). In contrast, the GLP-1 levels were substantially reduced in the 10% YBF-fed group, but not the 2.5% YBF group ($P < 0.05$).

Discussion

This study investigated whether YBF supplementation could counteract dyslipidemia, fatty liver disease, and dysregulation of metabolic hormones induced by HFD. YBF mitigated dyslipidemia by reducing TC, TG, and LDL levels in HFD-fed mice. Furthermore, YBF decreased oxidative stress and enhanced endogenous antioxidant activity in liver tissue. Notably, YBF also ameliorated HFD-induced histopathological changes in the liver, evidenced by a significant reduction in hepatic steatosis, inflammatory cells, abnormal sinusoids, and degenerated hepatocytes. Additionally, YBF counteracted HFD-induced hypersecretion of the FGF21 and GLP-1 hormones.

HFD has been shown to promote dyslipidemia, indicating by the elevation of TG, TG, and LDL levels in the blood [11]. Consistent with this, our current study found that a 12-week HFD treatment induced dyslipidemia in mice. In contrast, a higher dose of YBF improved plasma lipid profiles, suggesting its counteractive effect against HFD. Consuming a HFD could affect blood lipid profiles in both rodents and humans, influencing LDL levels [25]. Dietary cholesterol and saturated fats from HFD could elevate LDL levels [26]. Increased fat intake from HFD prompts the liver to produce more bile acids, using cholesterol, potentially raising blood LDL levels if dietary intake surpasses the liver's processing capacity

[25]. The liver might also boost its own cholesterol production in response to a HFD thereby elevating LDL [27]. Several plausible mechanisms could underlie the anti-hyperlipidemic effect of YBF. Firstly, YBF might directly bind to the dietary cholesterol from HFD in the gastrointestinal tract, limiting its translocation into the circulatory system. Consequently, blood cholesterol levels would be substantially reduced. Dietary fiber has been suggested to inhibit cholesterol absorption in the intestine [28]. Another study demonstrated that fiber could enhance bile acid excretion [29]. Thus, YBF might also bind to bile acids in the intestines, preventing their reabsorption and increasing their excretion. This action would stimulate greater cholesterol uptake by the liver, thereby reducing circulating cholesterol levels. Alternatively, YBF might promote an increase in SCFA production due to fiber fermentation by gut microbiota. A study on mice highlighted that dietary fiber, in the form of fructo-oligosaccharides, could lower plasma cholesterol levels by increasing SCFA production [27]. Furthermore, elevated SCFA levels have been found to reduce hepatic cholesterol production, leading to decreased plasma cholesterol levels [30]. Collectively, our findings support the idea that incorporating fiber into the diet could effectively manage HFD-induced dyslipidemia. Nonetheless, further studies are essential to ascertain whether YBF effectively binds to cholesterol, reduces bile acid reabsorption, and boosts SCFA production.

In addition to dyslipidemia, HFD can also induce pathological changes in the liver, leading to severe oxidative stress [31]. Accordingly, our current investigations revealed that HFD slightly increased liver mass and significantly elevated MDA (a marker of oxidative stress), while reducing CAT activity (an endogenous antioxidant) in liver tissue. In contrast, a higher dose of YBF reduced liver mass and MDA levels, while ameliorating CAT

activity levels in the liver. MDA is a by-product of lipid peroxidation, while CAT is an enzyme capable of counteracting free radicals, thereby reducing oxidative stress (32). A reduction in MDA level and an elevation in CAT activity level indicate YBF's ability to prevent lipid peroxidation and enhance endogenous antioxidants in the liver. It has been indicated that some types of fiber, especially soluble fiber, can exhibit antioxidant activity that can neutralize free radicals and reduce oxidative stress (33). Through this plausible mechanism, YBF may prevent HFD-induced MDA elevation while maintaining CAT activity. Additionally, YBF may also exert a counteractive effect against oxidative stress in the liver by improving lipid profiles. An increase in cholesterol levels indicates impaired lipid homeostasis, leading to oxidative stress, including in liver tissue (25). As previously indicated, YBF can manage plasma cholesterol levels against HFD, thereby reducing oxidative stress and improving endogenous antioxidant activity in the liver. Moreover, YBF may also contribute to managing liver oxidative stress by modulating the production of SCFAs, including propionate, butyrate, and acetate, which have been suggested to mitigate oxidative stress. For example, propionate has been reported to exert an antioxidant effect (34), and butyrate is also capable of alleviating tissue oxidative stress (35).

In addition to reducing oxidative stress and enhancing endogenous antioxidants, dietary fiber can also protect the liver from developing steatosis in the liver due to HFD (31). In our present study, mice fed with a higher dose of YBF had substantially fewer fat vesicles in their liver tissue, suggesting its mitigating effect against HFD-induced hepatic steatosis. This preventive effect could be associated with the capability if YBF to reduce the absorption of dietary fat from the intestine, thereby preventing excessive fat accumulation in tissues, including the liver. Moreover, an increase in SCFAs resulting from fiber fermentation in the gut might also contribute to lower hepatic steatosis under YBF supplementation. A report has indicated that SCFAs exert anti-hepatic steatosis effects under an HFD challenge (30). Additionally, YBF has been suggested to enhance the sensitivity of insulin in mice against HFD (21), while the resistance of insulin is one of the major causes of elevated fat accumulation leading to hepatic steatosis (36). Thus, YBF might also prevent hepatic steatosis by enhancing insulin sensitivity. The prevention of fat accumulation in liver tissue by YBF could also underlie the substantial reduction in liver weight observed in the mice treated with YBF compared to the other groups in our present study.

Accumulated oxidative stress and steatosis in the liver due to HFD intake have been shown to promote inflammatory responses and cellular degeneration (31). Similarly, this current study found that HFD led to an increase in macrophage infiltration and hepatocyte degeneration in the liver. Conversely, these pathological alterations were less pronounced with higher doses of YBF supplementation. In an in vitro study YBF exerted an immunomodulatory effect (7), suggesting that YBF was capable of preventing HFD-induced inflammatory responses in the liver. Moreover, YBF could also prevent inflammation and hepatocyte degeneration by reducing oxidative stress and sustaining endogenous antioxidant activity. Considering that dietary fiber could increase SCFAs production, thereby reducing inflammatory responses and cellular degeneration, it is possible that YBF may also indirectly exert a protective effect through these mechanisms. Furthermore, a previous study confirmed that a higher dose of YBF reduces the abundance of inflammatory-associated gut microbiota, namely *Mucispirillum* sp. (21), which may contribute to the reduction of inflammatory responses, including in liver tissue.

In this study, HFD caused hypersecretion of FGF21 and GLP-1. However, a higher dose of YBF effectively counteracted it. Both FGF21 and GLP-1 are involved in the regulation of metabolic homeostasis (13,14), while their hypersecretion is associated with dyslipidemia, liver pathology, and dysregulation of other metabolic hormones, including insulin, leptin, and glucagon (16,37,38). Taken together, our current results imply that YBF could potentially reduce the risk of these metabolic disorders by maintaining FGF21 and GLP-1 hormones at a physiological range. The precise mechanism by which a higher dose of YBF lowers FGF21 and GLP-1 levels against HFD remains to be defined by a future study. However, previous reports have suggested that dietary fiber could alter the gut microbiota composition, leading to the production of SCFAs and the regulation of metabolic hormones (29). Accordingly, SCFAs could promote the secretion of FGF21 and GLP-1, while counteracting resistance to these hormones (39). Hence, it is possible that YBF acts through the gut microbiota-SCFA axis to regulate FGF21 and GLP-1 levels.

The results of our study also highlight the importance of the YBF dose in exerting its counteractive effects against HFD. In some parameters, 2.5% YBF supplementation did not show substantial improvements compared to HFD alone. In contrast, at a higher dose (10%), YBF was more consistent in preventing the pathological outcomes of HFD. Therefore, the dose of YBF supplementation should be carefully considered when formulating diets to prevent metabolic disorders. Further studies using a wider range of YBF doses are required to determine the most effective dose in addressing diet-induced metabolic diseases.

While our findings shed light on the benefits of YBF against the detrimental effects of HFD, some limitations of this study should be appropriately considered. Firstly, the physicochemical characterizations of the YBF remain unknown, as this study did not include physical and

phytochemical analyses. Moreover, our present study did not conduct proximate analysis to clearly define the particular constituents of the diets supplemented with 2.5% and 10% YBF. Furthermore, this study did not determine the level of SCFAs, which is a key parameter to elucidate the functional relationship between fiber intake (YBF) and its physiological outcomes in overcoming the detrimental effects of HFD. The effect of YBF intake on other metabolic hormones, including ghrelin, glucagon, leptin, and neuropeptide Y, remains unclarified by this current study. In addition, the data on proinflammatory and anti-inflammatory cytokine levels in the liver and other tissues are also lacking in our study. Considerably, the physicochemical and proximate analysis of diets supplemented with YBF, along with molecular investigations elucidating the mechanisms of YBF in regulating lipid metabolism, liver function, and metabolic hormones, are required to deepen our understanding of the beneficial effects of YBF.

Conclusion

In conclusion, this current study demonstrated the ameliorative effects of YBF supplementation, especially at the 10% dose, in countering dyslipidemia, liver pathology including hepatic steatosis, and the hypersecretion of metabolic hormones FGF21 and GLP-1 caused by HFD. Therefore, incorporating YBF into the diet could be a valuable strategy for preventing the adverse effects of HFD, particularly metabolic diseases and associated health issues.

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Authors' contributions

Conceptualization: Putra Santoso.
Data curation: Rita Maliza.
Formal analysis: Putra Santoso.
Funding acquisition: Putra Santoso.
Investigation: Putra Santoso and Rita Maliza.
Methodology: Putra Santoso.
Project administration: Rita Maliza.
Resources: Rita Maliza.
Software: Rita Maliza.
Supervision: Putra Santoso.
Validation: Putra Santoso.
Visualisation: Rita Maliza.
Writing-original draft: Putra Santoso.
Writing-review & editing: Putra Santoso and Rita Maliza.

Conflict of interests

All authors declared no conflict of interest.

Ethical considerations

The experimental procedures in this study were approved by the Institutional Committee of Andalas University for research ethics and conduct (528/KEP.UA/022). The issues related misconduct, plagiarism, fabrication, and redundancy associated with the manuscript have been cleared.

Funding/Support

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