

BENEFICIAL EFFECT OF *PACHYRHIZUS EROSUS* FIBER AS A SUPPLEMENTAL DIET TO COUNTERACT HIGH SUGAR-INDUCED FATTY LIVER DISEASE IN MICE

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Abstract

Background and aims: Edible fiber isolated from the tuber of yam bean (*Pachyrhizus erosus*, Leguminosae) has been suggested to prevent the development of metabolic diseases caused by excessively consuming sugary foods. However, it is unclarified whether *P. erosus* fiber (PEF) is also capable of preventing liver diseases. This study aimed to determine the effectivity of PEF in counteracting the development of non-alcoholic fatty liver disease (NAFLD) caused by excessive intake of high-sugar diet (HSD). **Material and method:** 25% of PEF in HSD (w/w) was fed in BALB/c mice for eight weeks followed by subsequent morphological and histological examinations of the liver at the end of treatment in comparing with HSD alone. **Results:** We found that supplementation of 25% PEF sustained the normal morphological feature of the liver in HSD-fed mice. Moreover, PEF also prevented the development of microvesicles in the liver tissue, nuclear shrinkage, and hepatolysis as indicators of liver disease. Macrophages infiltration as a marker of liver inflammation was also less observed in PEF-treated mice. **Conclusions:** PEF could effectively elicit a beneficial effect against NAFLD caused by HSD. Hence, PEF is suggested to be used as a potent supplemental diet to preclude the metabolic diseases caused by HSD.

key words: diabetes, edible fiber, hepatolysis, non-alcoholic fatty liver disease, obesity

Background and aims

The prevalence of liver diseases including non-alcoholic fatty liver disease (NAFLD) is markedly increasing all over the world [1]. NAFLD spectrum includes the non-alcoholic fatty liver, hepatic cirrhosis, hepatic carcinoma, and non-alcoholic steatohepatitis with high morbidity and mortality [2]. Excessive intake of

high-sugar diet (HSD) and high-fat diet (HFD) has been attributed to the development of NAFLD both in animal models and humans [3,4]. Chronic HSD and HFD treatments elevated lipid accumulation, inflammation, fibrosis and cirrhosis in the liver [5]. A previous report also has demonstrated that HFD could progressively enlarge the vacuole in the liver as an indicator of hepatic steatosis [6]. The salient

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increase of hepatic steatosis is due to a higher rate of lipid peroxidation in the liver tissue that commonly observed in individuals with obesity and associated metabolic diseases [7].

We have previously revealed that HSD-induced metabolic diseases, including obesity and diabetes in mice, could be precluded by supplementing high-fiber diet isolated from the tuber of yam bean (*Pachyrhizus erosus*) [8]. This fiber, namely *Pachyrhizus erosus* fiber (PEF), was shown to be effective in preventing the increase of white adipose tissue mass and glucose intolerance as indicators of metabolic disturbance. Furthermore, consuming this fiber exerted a minimum effect on the rhythms of food and water intake. However, it is unelucidated whether PEF could also be effective in preventing the development of liver diseases particularly NAFLD. Another report has shown that fiber isolated from bamboo shoot could significantly diminish the HFD-induced an increase of liver weight and its lipid content such as triglyceride and total cholesterol [9]. An experiment using fiber from sugarcane also demonstrated that dietary fibers are capable of attenuating hyperaccumulation of hepatic lipid by enhancing AMPK signaling in the liver [10].

In this current investigation, we attempted to unravel the beneficial effect of yam bean fiber (PEF) in counteracting the development of fatty liver disease caused by consuming high-sugar food. We used normal mice fed with diet containing high sucrose to induce NAFLD and, at the same time, supplement it with 25% of PEF. Our finding suggests that PEF could promote a hepatoprotective effect against sugar-induced fatty liver disease.

Material and method

Animal model

In this study, we used the Bagg and Albino (BALB/c) mice strain (n = 24, 2 months old,

male with body weight ± 25 g) provided by UD WISTAR Yogyakarta. During a 7-day acclimatization, each mouse was reared in a single cage (1 mouse/cage) and fed with standard diet and tap water *ad libitum* in the animal room with normal light and dark cycle and regulated temperature (25-27°C). The animal maintenance as well as experimental procedures performed in this research were in accordance with the standard guidelines for Animal Care and Use of Andalas University and in line with the recommendation of Declaration of Helsinki.

Preparation of PEF

The tubers of yam bean were freshly collected from the local farmer in District of Pariaman (West Sumatra). The fiber of yam bean (PEF) was prepared by following the protocol as we described in previous report [8]. Briefly, after being peeled, the tubers were washed with distilled water and subsequently sliced and grated using a manual grater for 10 min. Furthermore, the sample was suspended into distilled water overnight in an isolated jar. On the next day, the fiber was isolated from the suspension and steamed for 40 min. Then, the sample was dried up in the oven (67-70°C) for 16 h. The dried fiber was ground to be powder and subsequently used for the experiment. For the purpose of proximate analysis, the crude yam bean powder was also prepared by grinding the tubers and dried it up subsequently in the oven without any further processing.

Proximate analysis of PEF

The proximate analysis to determine the protein, fat, total carbohydrate, simple sugar content, ash, and water content of PEF and crude powder of yam bean was performed by following the standard protocols [11].

Experimental design

In this study, the animals were equally divided into two groups (n =12 for each group) namely (a) high-sugar fed group (HSD) and (b) HSD + *P. erosus* fiber- fed group (HSD + PEF 25%). In HSD group, animals were fed with a diet composed of 70% normal diet (BP2 diet, Java Comfed Jakarta) and 30 % solid sucrose. In HSD + PEF group, animals were fed with a diet composed of 45% normal diet, 30% solid sucrose, and 25% *P. erosus* fiber. The diets were given to the animals *ad libitum*. The treatments were performed continuously for 8 weeks while the food and water were changed once a day to sustain its quality. In this experiment, we did not include the normal control group (fed only with a normal diet, BP2) since that we have previously performed a preliminary study revealing that such diet did not cause any structural damages and morphological alterations in the liver of mice.

Body weight measurement

Each mouse was subjected for body weight measurement using a digital scale. Furthermore, it was photographed to depict the fat accumulation both dorsal and ventral views.

Morphological and histological examination of liver

The mice were sacrificed after the treatment and the livers were collected gently,

photographed and subjected for weight measurement. Furthermore, the samples were fixed in formaldehyde 10% overnight. The fixed liver tissues were processed for histological examination and hematoxylin-eosin staining by a standard protocol as explained elsewhere [12]. The tissue slides were examined under microscope (Olympus, Tokyo Japan) to determine the structural alterations and the representative samples were photographed. We used a descriptive method to describe the structural alterations of the liver instead of systematic histopathological grading.

Statistical analysis

The quantitative data including the liver weight, liver index and body weight were analyzed using a Student t-test with $p < 0.05$ was considered as significant.

Results

In this present investigation, we firstly determined the nutrient content of PEF and compared it with the crude yam bean powder (contained whole part of the tuber except the skin) by means proximate analysis. As shown by the data (Table 1), the isolation process markedly reduced the starch content in PEF as compared with crude powder by 85.95%. Importantly, the isolation procedures conserved the protein and fat content in PEF and reduced the simple sugar.

Table 1. Nutrient contents of crude powder and isolated fiber of *P. erosus*

No	Parameters	Content (% per gram)	
		Crude powder	Fiber (PEF)
1	Protein	2,362	3,326
2	Fat	0,349	0,632
3	Starch	55,763	7,833
4	Sugar	3,272	1,291
5	Ash	0,904	1,615
6	Water	5,295	1,543

We subsequently examined the effectivity of PEF in counteracting the HSD-induced hepatic

steatosis. The supplementation of PEF tended to reduce the liver weight and liver index in mice

consuming HSD (Fig. 1A and B) but it was not significantly different statistically. However, the gross morphological examination revealed that the supplementation of PEF could prevent the color alteration of the liver in HSD-treated mice (Fig. 1C for HSD alone vs. Fig. 1D for HSD + PEF 25%), suggesting the counteractive effect of PEF against liver disease. Furthermore, we performed a microscopic observation on fixed liver tissues to investigate the structural changes of liver. As depicted in Fig. 2A and C, chronic HSD treatment induced the development of fatty liver disease as indicated by the formation of microvesicles in the liver. Inversely, the microvesicles were less observed in the liver of HSD + PEF 25%-fed mice (Fig. 2B and D). A further observation using higher magnification on the liver tissue (Fig. 3) revealed that HSD treatment caused cellular damages including shrinkage, picnotic and lysis (Fig. 3A, B), while

those cellular alterations were lack in the liver of HSD + PEF 25%-treated mice.

Additionally, as expected, we also found that HSD-treated mice developed obesity as indicated by excessive abdominal fat and a significant increase in body weight. Otherwise, the HSD + PEF 25%- treated mice had normal abdominal fat and lower body weight (Fig.4A-C).

Discussion

Our present data demonstrated that a simple aqueous isolation of edible fiber from yam bean yielded a high concentration of fiber with minimum starch content. Moreover, this isolation procedure did not cause an apparent reduction in protein and fat content. Therefore, the isolated edible fiber (PEF) was nearly free of digestible carbohydrate but conserved protein and a small amount of fat.

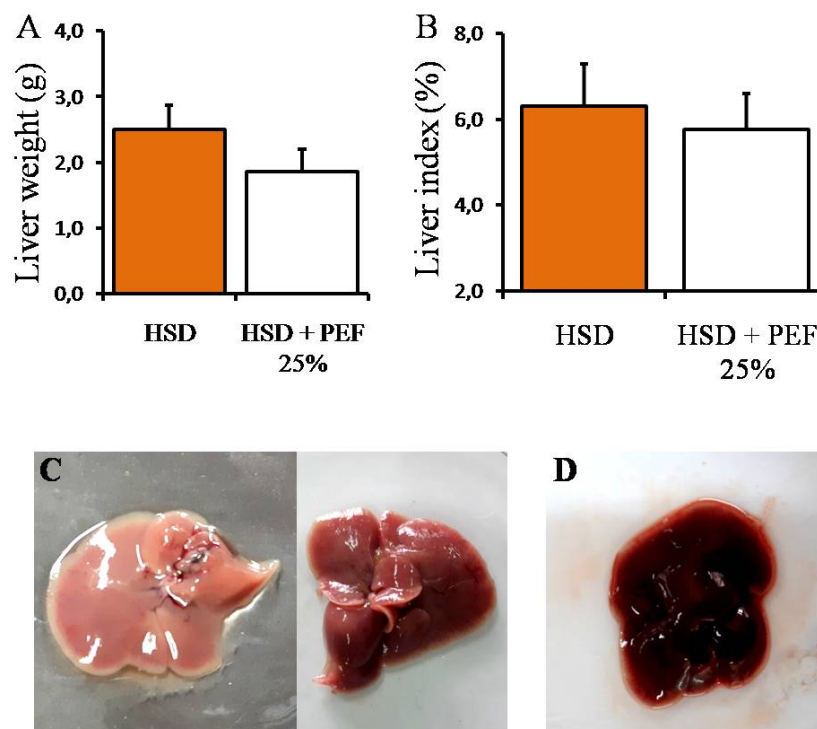


Figure 1. Liver weight and its morphological features in mice treated with high-sucrose diet (HSD) and HSD with 25% *Pachyrhizus erosus* fiber (PEF) for 8 weeks. (A) Liver weight measured at the end of treatment, (B) Liver index representing the percentage of its weight against body weight, (C) Morphological features of the liver of HSD-treated mice, and (D) HSD + 25% PEF-treated mice.

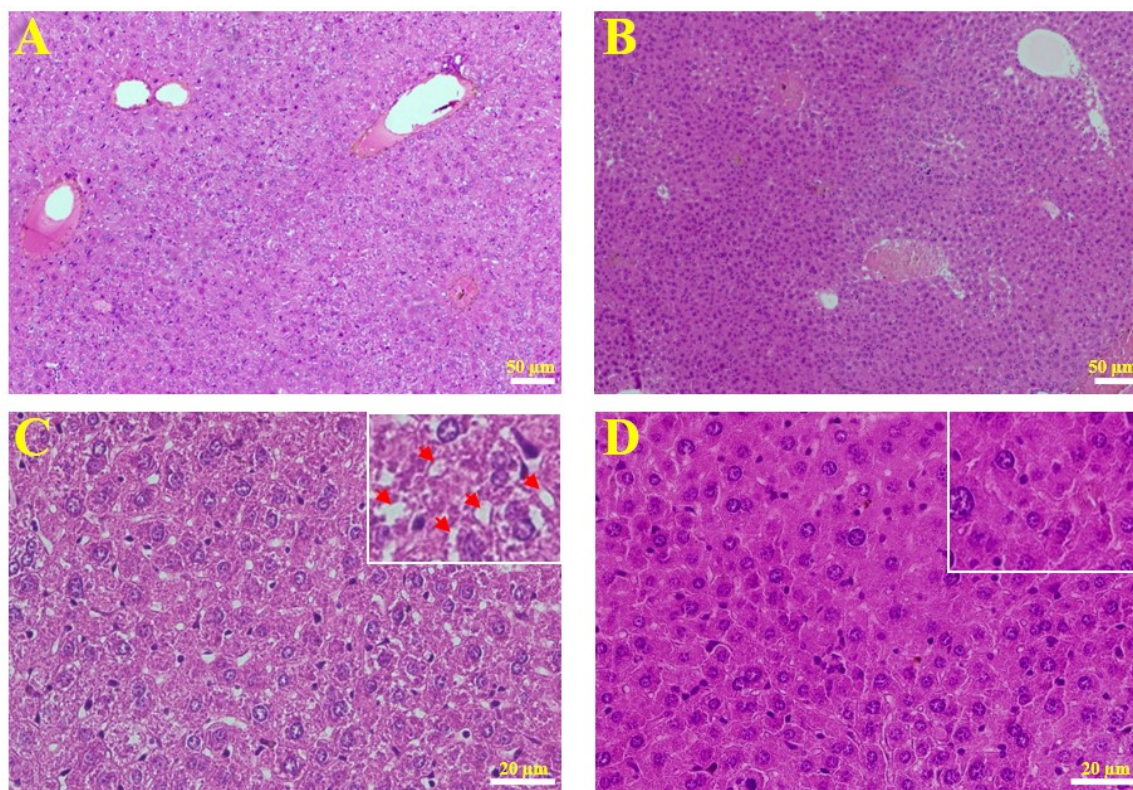


Figure 2. Representative photomicrograph of liver tissue in mice treated with HSD and HSD in combination with 25% of PEF for 8 weeks. (A, C) Liver tissue of HSD-treated mice depicting the microvesicle as an indicator of steatosis, (B, D) Liver tissue of HSD + 25% PEF showing minimum microvesicles. The white squares in C and D represent the higher magnification of tissue. Red arrows in C indicate the microvesicles. Tissues were stained with HE. Scale in A and B = 50 μm , C and D = 20 μm .

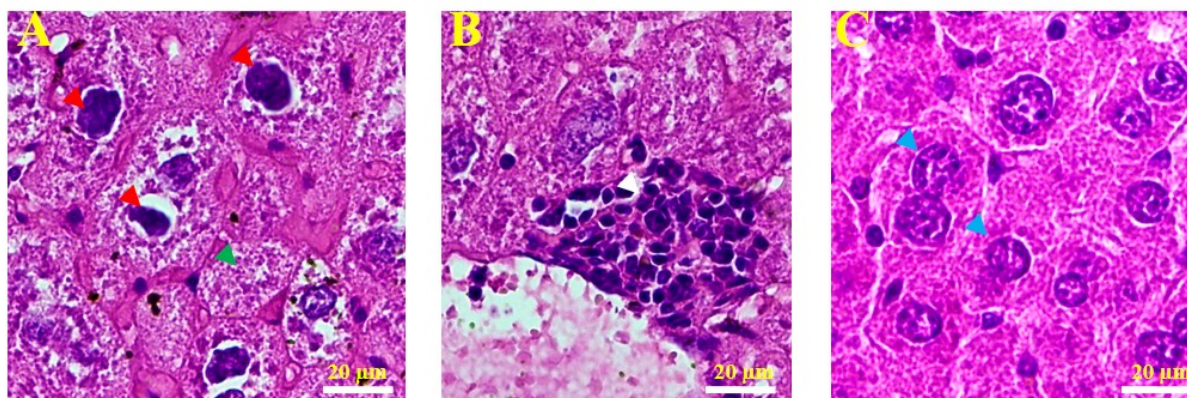


Figure 3. Representative photomicrograph of liver tissue depicting the cellular features in high magnification. (A) The hepatocytes of mice treated with HSD alone showing the nuclear shrinkage (indicated by RED arrows) and lysis (green arrow), and (B) the macrophage infiltration in liver tissue (white arrow) of HSD group (C) normal features of hepatocytes in mice fed with HSD + PEF 25% with clearly visible normal nuclei (blue arrows) and none cellular damage. Scale bar = 20 μm .

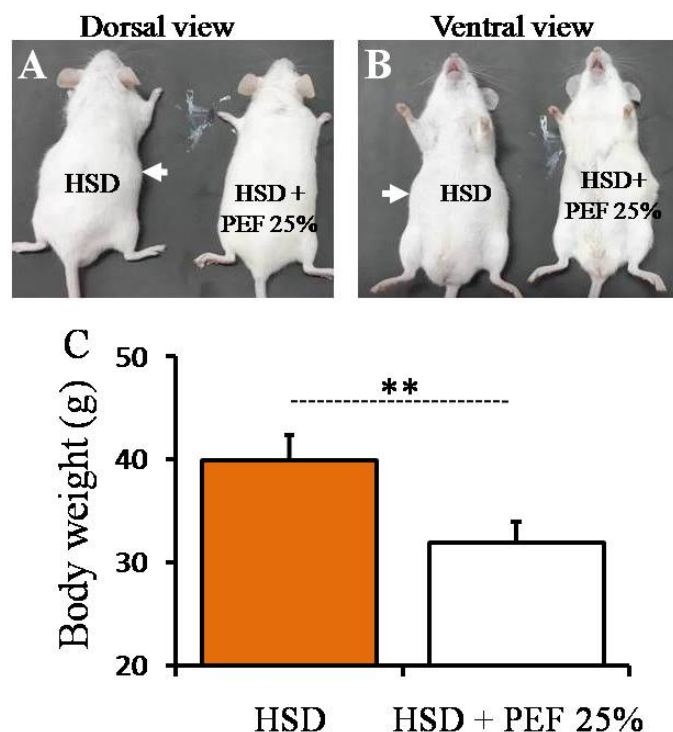


Figure 4. Morphological features and body weight of mice after 8 weeks of treatment. (A, B) the dorsal and ventral views of HSD-treated mice and HSD with 25% of PEF-treated mice, (C) Body weight of mice at the end of treatment. **) indicates the significant difference ($P < 0.05$) by student t- test. White arrows in A and B indicate the increase of abdominal fat.

We have previously reported that PEF 25% was effective to prevent the surge of blood glucose and adiposity in mice fed with HSD [8]. Extendedly, in this recent study, we revealed that supplementation of PEF could also effectively counteract the development of NAFLD. It has been reported that either complex or simple sugar could profoundly increase the hepatic steatosis [13]. Excessive sugar intake might increase lipid accumulation in the liver tissue and could lead to hepatocellular damages as commonly observed in NAFLD [2]. Our present findings suggest that such damages could be precluded by the high-fiber intake.

There are several plausible mechanisms of PEF to counteract the development of sugar-induced NAFLD. First, high fiber intake could slow down the gastric emptying during feeding thereby delayed and reduced sugar absorption in the intestine [14-16]. Hence, the lesser the

sugar absorption, the lower the risk of developing NAFLD. Secondly, the ingestion of PEF could trigger the release of gastrointestinal hormones including ghrelin-like peptide 1 (GLP-1) [17]. The increase of GLP-1 secretion could subsequently activate the neuronal signaling pathway in the hypothalamus that eventually increases energy expenditure [17]. This mechanism will indirectly prevent the lipogenesis and its accumulation in the tissues including liver tissues. Third, the PEF could be fermented by the microbiota in the gastrointestinal tract particularly in the large intestine to be fatty acids (short-chain fatty acids, SCFAs). It has been reported that SCFAs including butyrate, propionate and acetate could exert a beneficial effect against liver damages [18]. Another plausible mechanism that could underly the prevention of sugar-induced NAFLD is the protective effect elicited by some bioactive

compounds in the PEF including inulin, vitamin C and flavonoids [19]. It has also been reported that yam bean contains antioxidants including terpenoids and saponin that might function as a hepatoprotector [20]. Since that liver damage is caused by elevated oxidative stress as well as aberrant inflammatory responses [21], thus PEF and its antioxidant properties could both direct and indirectly diminish such degenerative process. However, further extensive investigations should be done in the future to clarify the assumptions.

Conclusions

In conclusion, our current research revealed that supplementation of PEF could effectively counteract the development of NAFLD

symptoms in mice fed with sugary food (HSD). PEF minimized the microvesicle formations in the liver, sustained the normal gross morphological features of the liver and prevented HSD-induced hepatocellular alterations. Therefore, PEF is recommended to be formulated as a supplemental diet that could counteract the detrimental impacts caused by excessive consumption of sugary foods.

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