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Protective effects of functional powdered beverage containing Cassumunar ginger (*Zingiber cassumunar* Roxb.), soybean (*Glycine max*), and cinnamon (*Cinnamomum burmannii*) against hyperlipidemia and injuries of liver and kidney in high-fat-diet rats

Nurkhasanah Mahfudh[a,*], Ika Dyah Kumalasari[b], Nanik Sulistyani[a], Candra Azzahra[a], Salma Dewina Salimah[a], Suryati Suryati[a], Zainul Amiruddin Zakaria[c]

[a] Faculty of Pharmacy, Universitas Ahmad Dahlan, Janturan, Yogyakarta 55164 Indonesia
[b] Faculty of Industrial Engineering, Universitas Ahmad Dahlan, Bantul, Yogyakarta 55166 Indonesia
[c] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah 88400 Malaysia

[*]Corresponding author, e-mail: nurkhasanah@pharm.uad.ac.id

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ABSTRACT: A high-fat consumption can cause hyperlipidemia, which is marked by an increase of total cholesterol, triglycerides, and low-density lipoprotein (LDL); and a decrease of high-density lipoprotein (HDL) in serum. This condition can lead to various degenerative diseases. This study aimed to observe the effects of functional powdered beverage (FPB) on levels of cholesterol, triglyceride, blood urea nitrogen (BUN), and creatinine; SGOT and SGPT activities; and liver histopathology in rats given a high-fat diet (HFD). The FPB was made of *Zingiber cassumunar* rhizome, soybean, and cinnamon. Test animals were divided into six groups: normal, negative control, positive control, and three treatment groups. While the normal group was received only a standard diet, the other five groups were received HFD along the studies. In addition, the three treatment groups were treated with different doses of FPB of 1000, 1500, and 2000 mg/kg BW, respectively. Biochemical parameters were measured with UV-Vis spectrophotometry, and histopathological observations used hematoxylin and eosin (H&E) staining. The data were analyzed statistically at a 95% confidence level using SPSS. In comparison to the negative control, the results demonstrated a statistical reduction in cholesterol, triglycerides, BUN, and creatinine levels ($p < 0.05$), indicating the effect of the functional powdered beverage as an antihyperlipidemic agent and protection for hyperlipidemia-associated kidney injuries. SGOT and SGPT activities were also lowered significantly ($p < 0.05$) in comparison to the negative control, suggesting a hepatoprotective effect of the beverage in HFD. Improved liver histology and decreased hepatosomatic index (HI) in the three treatment groups attested to the beverage's hepatoprotective activity, with the best pharmacological effects obtained at 2000 mg/kg BW.

KEYWORDS: antihyperlipidemic, hepatoprotective, *Zingiber cassumunar*, soybean, cinnamon, cholesterol, triglyceride, SGOT, SGPT

INTRODUCTION

Hyperlipidemia is a health problem with increasing incidence and prevalence worldwide. It occurs due to an excess of fats in the form of lipids, cholesterol, and triglycerides in the blood. Therefore, lifestyle choices incorporating a high fat intake can induce hyperlipidemia.

The rhizome extract of *Zingiber cassumunar* Roxb., known as Cassumunar ginger, or *bangle* in Indonesia, has antioxidant activity that can inhibit lipid oxidation [1]. Administering the extract at a dose of 400 mg/kg BW increases superoxide dismutase (SOD) enzyme activity significantly ($p < 0.05$) compared with hyperlipidemic control. In rats, increased antioxidant enzyme activity can prevent blood lipid elevation after high-fat diet treatment [2]. *Z. cassumunar* extract also has hepatoprotective activity suppressing hepatic lesion damage induced by carbon tetrachloride (CCl_4) [3].

Soy foods and supplementation effectively prevent obesity and heart disease by reducing serum lipid profile parameters, including triglycerides and HDL [4]. In postmenopausal obese rats, 150 mg/kg BW per day of soy isoflavone extract has also been reported as a hepatoprotective agent [5] that significantly reduces fatty liver and plasma biomarkers of liver damage ($p < 0.05$), in comparison to high-fat diet (HFD) control [6]. In addition, soy supplementation is known to improve kidney function in hypercholesterolemic conditions [7, 8].

Phytochemical constituents of cinnamon (*Cinnamomum burmannii*), including trans-cinnamaldehyde and cinnamic acid, are sources of antioxidants that can prevent free radical formation, eliminate radicals before causing damages, repair oxidative damages, and reduce lipid peroxidase activity [9]. In test rats, these antioxidant properties,

Table 1 Composition of FPB.

Ingredient	Weight (g)
Cassumunar ginger (*Z. cassumunar* Roxb.) rhizome	37.5
Sugar	37.5
Soybean (*G. max L.*), powdered	23
Cinnamon powder (*C. burmannii*)	2
Total	100

introduced through 320 mg/kg BW of cinnamon extract, are responsible for repairing paracetamol-induced liver injuries by significantly lowering cell damage parameter scores ($p < 0.05$) and SGOT and SGPT levels in comparison to control group [10].

A functional beverage made of Cassumunar ginger rhizome, cinnamon, and soybean (*Glycine max*) has been previously formulated by [11] as an instant drink with an antioxidant effect. The combination of various spices in functional beverages allows interactions between their active ingredients, which are expected to have a synergistic effect of producing higher activities than a single ingredient and cover the weakness of other components. A previous study presented the evidence of antioxidant effect of the functional beverage in *in vitro* level, and the present study was intended to examine the potency of functional powdered beverage (FPB) formulated from Cassumunar ginger rhizome, cinnamon, and soybean in suppressing lipid elevation in HFD rat and observe its effects of protecting liver and kidney in the hyperlipidemic rats *in vivo*.

MATERIALS AND METHODS

Plant collection

Cassumunar ginger rhizomes, soybeans, and cinnamons were purchased from a local market (Beringharjo Market), Yogyakarta, Indonesia. All samples were authenticated at the Laboratory of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, with the plant certificate no. 189/Lab.Bio/B/VI/2021.

Preparation of FPB

The optimum formula for the FPB was obtained from a previous study [11]. The formular, which had the best organoleptic acceptance scores given by panelists, was presented in Table 1.

Preparation of a HFD

In order to create a HFD feed, 300 g of regular feed was combined with 100 g of butter, 10 g of beef fat, 20 g of chicken egg yolk, and 0.05% propylthiouracil (PTU). These ingredients were mashed and ground in a grinder and then shaped into the size of a standard feed. Afterward, the feed was dried in an oven at 50 °C for 3 days [12].

Animal handling

Thirty 2–3-month-old male Wistar rats, each weighing 150–250 g, were obtained from the Laboratory of Muhammadiyah University of Yogyakarta. The animals were kept in a sufficiently lit room with a 12:12 light-dark cycle, adequate ventilation, and humidity maintained at 45–50%. They were fed a standard diet for a week with ad libitum access to water for 7 days to acclimatize them before the experiment [13]. The Study Ethics Committee of Universitas Ahmad Dahlan had granted consent for the use of animals in this research method, approval number 012106034.

Experimental design

Six groups of five test animals were randomly created from the 30 Wistar rats. The sample size was calculated using Federer's formula. The six groups and their respective treatments during the test were as follows: the normal group (one set), standard diet food; the negative control group (one set), HFD only; the positive control group (one set), oral administration of HFD and Nutrive Benecol® twice a day at 9 ml/kg BW; and the treatment group (three sets), oral administration of HFD and FPB at 1000, 1500, and 2000 mg/kg BW of the individual sets, respectively. During the experiment, all the groups were given 15 g of their respective feed per feeding time and ad libitum access to water for 28 days to observe the occurrence of hyperlipidemia. Nutrive Benecol and FPB were given from day 15 to 28. The combination of HFD and PTU has been reported to increase the lipid profile in animal models [14], and the Nutrive Benecol® has been proven effective in reducing cholesterol levels in a randomized, double-blinded clinical trial [15].

Blood collection and preparation of rat livers

At the end of treatment, on day 29, blood was collected through the orbital sinus and then centrifuged to separate serum and plasma. The serum samples were tested for levels of triglycerides, cholesterol, blood urea nitrogen (BUN), and creatinine; and activities of glutamic oxaloacetate transaminase (SGOT) and glutamic pyruvic transaminase (SGPT). Then, the test animals were sacrificed and dissected, and the liver was removed, weighed, and fixed in a 10% formaldehyde solution for histopathological examination.

Cholesterol and triglyceride tests

Two photometric enzymatic tests were used in the analyses: CHOD-PAP (Cholesterol Oxidase-Peroxidase Aminoantipyrine) for cholesterol level and GPO-PAP (Glycerol-3 Phosphate Oxidase-Phenol Aminoantipyrine) for triglyceride level. In both tests, a DiaSys® reagent kit with was used to measure the photometric absorbance at 546 nm.

Measurement of BUN and creatinine levels

BUN and creatinine levels were quantified using the DiaSys® reagent kit and UV-Vis spectrophotometry (UV 1900i Shimadzu, Japan), BUN was determined with a glutamate dehydrogenase (GLDH) assay and read at 340 nm, while the creatinine level was measured using Jaffé's method and read at 492 nm.

Measurement of SGOT and SGPT activities

The DiaSys® reagent kit, an enzymatic method, was used to determine SGOT and SGPT activities. Both were measured with UV spectrophotometry at 365 nm. The two activities indicate the state of liver damage. The samples were tested for protective effects from the percentage of decrease in SGOT and SGPT activities of the treatment groups compared with the negative control.

Hepatosomatic index (HSI) calculation

After blood collection, test animals were sacrificed with CO_2 gas, and the lower abdomen was dissected to remove the liver. Afterward, the liver was rinsed with physiological saline and weighed. The ratio of liver weight (g) to body weight (g) was used to determine the HSI [16].

Hepatic steatosis observation

The liver tissue was stained with H&E and then observed under a microscope at 200× and 400× magnifications. In addition, the sample was divided into four sections, and any changes in the tissue's microanatomical structure within each section were scored using the modified Mordue method [17].

Statistical analysis

The mean and standard deviation (SD) were used to express quantitative data. For parametric data, one-way analysis of variance (ANOVA) and a post-hoc Tukey test were used; for non-parametric data, Kruskal-Wallis and Mann-Whitney tests were used. Data were considered statistically significant at $p < 0.05$.

RESULTS

Table 2 shows weight changes in the test rats before and after 28 days of the experiment. Compared with the normal group (standard feed), the HFD in the negative control group substantially increased the rat's body weight. Also, it was found that the FPB added to the HFD feed decreased body weight significantly ($p < 0.05$). In addition, HFD increased cholesterol and triglyceride levels. The cholesterol level of the normal group (45.78±6.09 mg/dl) was significantly lower than that of the HFD treated negative control (147.36±10.29 mg/dl). This data confirmed previous research finding that HFD treatment could be used for hyperlipidemic treatment for hyperlipidemic model in animal [22]. Fig. 1 shows the effects of giving FPB on the cholesterol and triglyceride levels of rats with HFD. The negative control had significantly ($p < 0.05$) cholesterol and triglyceride levels higher than the normal, the positive control, and the three treatment groups. The lowest cholesterol and triglyceride levels were obtained from 2000 mg/kg BW of FPB; both figures were statistically different ($p < 0.05$) from the results of giving Nutrive Benecol (the positive control group).

According to a prior study, hyperlipidemia accelerates the development of kidney injury. Lipids might cause damages to the glomeruli and tubulointerstitial tissue [19]. Fig. 2 shows the BUN and creatinine test results as indicators of kidney function of rats fed with standard feed, HFD, Nutrive Benecol, and FPB. The negative control (HFD) showed significantly high BUN and creatinine levels ($p < 0.05$) compared with the normal group (standard feed). The lowest BUN and creatinine levels were achieved in the HFD rat treated by FPB at dose of 1500 and 2000 mg/kg BW. These findings were statistically different ($p < 0.05$) compared with the negative control (given HFD only). For liver function, SGOT and SGPT activities were statistically high ($p < 0.05$) in the negative control compared with the normal group (Fig. 3). In contrast, those fed with HFD and FPB showed significantly low SGOT and SGPT activities ($p < 0.05$) (Fig. 3), indicating the protective effect of FPB.

Hyperlipidemia can trigger lipids accumulation in the liver. A high-fat diet increases the amount of fat absorbed in the gastrointestinal tract, which will be transported to adipose tissue or extrahepatic tissue. Changes in liver weight are an indicator of fat accumulation [20]. The liver weight, the body weight, and the hepatosomatic index of the test animals were presented in Table 3.

To confirm the excess of fat, further histological observations of liver sections were conducted by H&E staining on all six test groups, as shown in Fig. 4. The microanatomical picture of liver cells in the normal control group (standard feed without special treatment) showed a normal hepatic cell structure, a typical ratio between cytoplasm and nucleus, no fat degeneration, and no necrosis (Fig. 4a). In contrast, fatty degeneration was found in the liver sections of rats treated with HFD, HFD + Nutrive Benecol, and HFD + 1000 mg/kg BW of FPB observed at 400× magnification (Fig. 4b–d). As indicated by white cells, fat-filled vacuoles occupied the cell's center, pushing the nucleus to one side. Fatty degeneration could occur due to the continuous administration of HFD and the inability of cells to remove triglycerides accumulating in the blood [21].

Fig. 4 shows that the liver in negative group (HFD) experiencing local fatty degeneration showed a vac-

Table 2 Effects of FPB on body weights in rats with a high-fat diet (expressed as mean ± SD).

Group	Average body weight		Weight change (g)
	Beginning (g)	End (g)	
Normal	181.00 ± 4.21	182.54 ± 2.45	1.54 ± 0.47*
Negative control	174.24 ± 5.86	192.46 ± 9.46	18.22 ± 7.48
Positive control	268.68 ± 12.37	263.52 ± 15.49	−5.16 ± 7.04*
1000 mg/kg BW	184.26 ± 3.47	185.16 ± 7.19	0.90 ± 5.41*
1500 mg/kg BW	207.88 ± 13.62	201.90 ± 14.91	−5.98 ± 8.43*
2000 mg/kg BW	227.48 ± 6.13	217.72 ± 7.55	−9.76 ± 5.58*

* = significantly different from negative control.

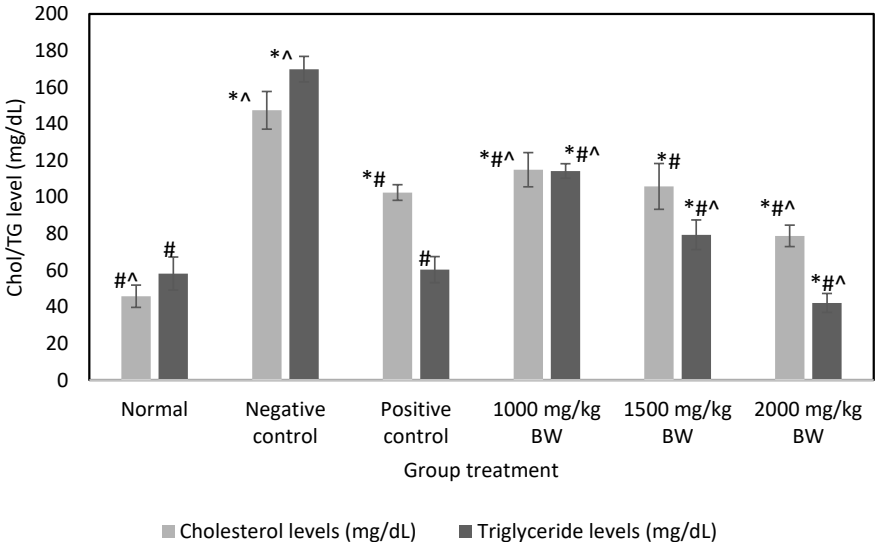

Fig. 1 Effects of FPB on cholesterol and triglyceride levels in HFD rats (in group average, mean ± SD). * statistically different from the normal group, ($p < 0.05$); # statistically different from the negative control, ($p < 0.05$); ^ statistically different from the positive control, ($p < 0.05$.

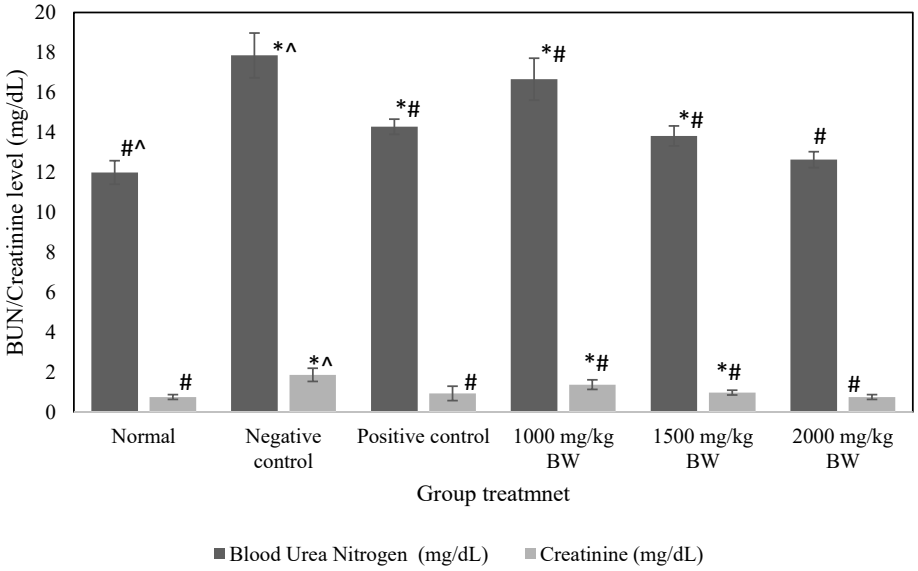

Fig. 2 Effects of FPB on the renal function of rats with a high-fat diet (in group average, mean ± SD). * statistically different from the normal group, ($p < 0.05$); # statistically different) from the negative control, ($p < 0.05$); ^ statistically different from the positive control, ($p < 0.05$).

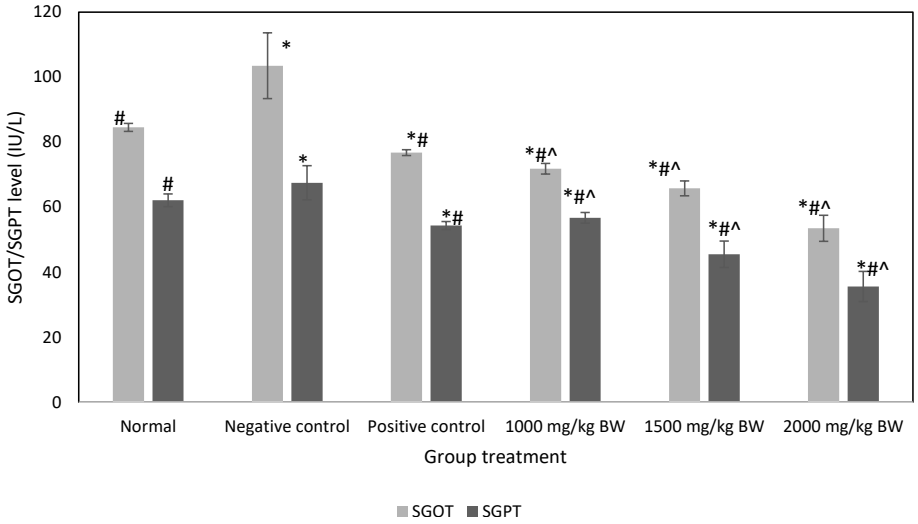

Fig. 3 Effects of FPB on liver function (SGOT and SGPT) in rats treated with a high-fat diet (in group average, mean ± SD). * statistically different from the normal group, ($p < 0.05$); # statistically different from the negative control, ($p < 0.05$); ^ statistically different from the positive control, ($p < 0.05$).

Table 3 Hepatosomatic index values of rats given high-fat feed, Nutrive Benecol, and the FPB at different doses (in group average, mean ± SD).

Group	Body weight (g)	Liver weight (g)	Hepatosomatic index
Normal	182.54 ± 2.45	6.92 ± 1.03	3.79 ± 0.58#
Negative control	192.46 ± 9.46	8.00 ± 0.64	4.17 ± 0.46^
Positive control	263.52 ± 15.49	8.45 ± 0.91	3.20 ± 0.22*#
1000 mg/kg BW	185.16 ± 7.19	7.63 ± 0.79	4.13 ± 0.47^
1500 mg/kg BW	201.90 ± 14.91	6.17 ± 0.46	3.06 ± 0.20*#
2000 mg/kg BW	217.72 ± 7.55	6.45 ± 0.62	2.96 ± 0.28*#

* = statistically different from the normal group, ($p < 0.05$); # = statistically different from the negative control, ($p < 0.05$); ^ = statistically different from the positive control, ($p < 0.05$).

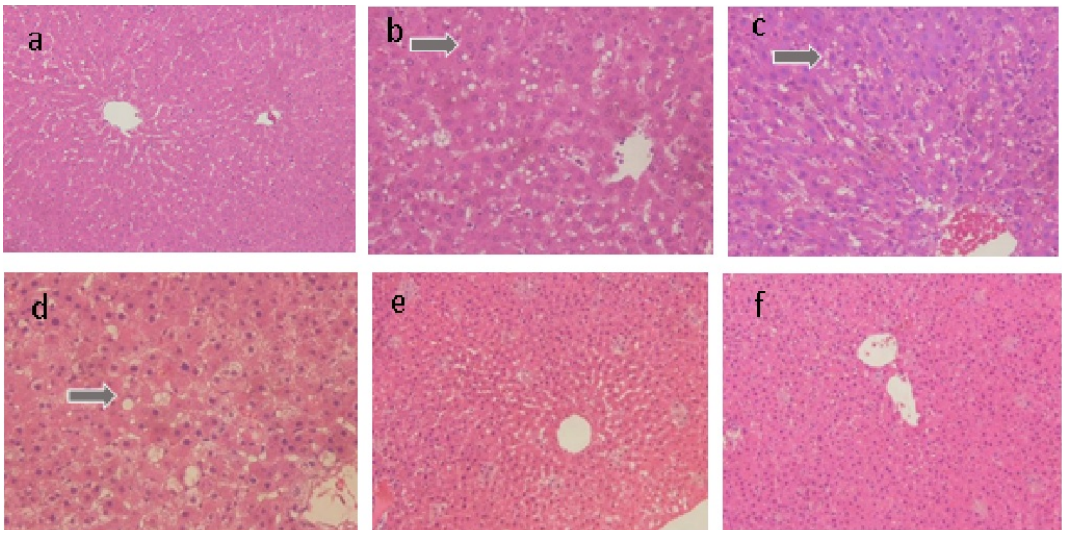

Fig. 4 Liver sections of rats given different feeds and treatments stained with H&E: (a), standard feed; (b), HFD; (c), HFD + Nutrive Benecol; (d), HFD + FPB at 1000 mg/kg BW; (e), HFD + FPB 1500 mg/kg BW; and (f), HFD+FPB 2000 mg/kg BW. Observations were done at 200× magnification.

Table 4 Microscopic scoring of liver in rats receiving different feeds and treatments.

Group	Microscopic scoring
Normal	0
Negative control	1.3
Positive control	1
1000 mg/kg BW	1
1500 mg/kg BW	0
2000 mg/kg BW	0

uole appearing with distinct boundaries; however, there was no necrosis. While the positive group (HFD + Nutrive Benecol) showed damages to the cell structure, called parenchymatous degeneration, as characterized by the swelling or enlargement of cytoplasm (ballooned cells). Compared to fatty degeneration, parenchymatous degeneration is a mild liver disorder.

Table 4 shows the microscopic scoring of all six test groups. Microvesicular steatosis in the negative control group (HFD) showed a fatty degeneration score of 1.3. Treatment with 1000 mg/kg BW FPB had the same score of 1 as the positive control (HFD + Nutrive Benecol); but for both 1500 and 2000 mg/kg BW FPB treatments, the microscopic score was 0.0.

DISCUSSION

Frequent consumption of high-fat food as one's lifestyle has become more common worldwide. An imbalance between calorie intake and output can cause obesity, hyperlipidemia, and other metabolic syndromes. Metabolic syndromes are risk factors for a variety of illnesses, including cardiovascular disease, type 2 diabetes mellitus, and certain types of cancer. This research found that a HFD made from different sources of saturated fat, namely egg yolks, butter, and beef fat, increased body weight of test rats (see Table 2). Consuming foods containing saturated fat and cholesterol could cause cholesterol and lipid buildup in the blood [22]. Saturated fat in foods stimulates the liver to produce cholesterol and, hence, raise blood cholesterol level. Moreover, the increase in body weight caused by high blood cholesterol and triglycerides (induced by HFD) confirmed the positive correlation between obesity and fat intake reported in previous studies [23]. Oxidation and deposition of high fat intake raised body weight and obesity incidence.

This study showed that 28 days of HFD could increase cholesterol and triglyceride levels in rats, compared with the normal control group (Fig. 1). Elevated cholesterol and triglyceride levels could be associated with oxidative stress due to increased ROS and decreased antioxidant enzyme activities. Oxidative stress potentially damages the cells of organs. Prolonged exposure of cells to ROS leads to imbalances and disturbances of lipid metabolism [24]. Previous studies also reported increased blood lipid levels and body weight after a high-fat diet [25, 26]. Curcumin, one of the major compounds in *Z. cassumunar* rhizome, has an antioxidant activity through the mechanism of increasing antioxidant enzyme activities, including glutathione-S-transaminase (GST) and glutathione peroxidase (GPx) [27].

Mobilization of fat from blood and adipose tissue to the liver is responsible for the risk of diet-induced fatty liver disease or steatosis. Lipid concentrations in an organ are regulated by the balance of one or more processes in lipid transport to the liver, including absorption, synthesis, oxidation, and secretion [28]. The high hepatosomatic index (HI) after HFD confirmed lipid accumulation [20]. ROS produced by the HFD and the excessive accumulation of fat in the body could induce damage to hepatocytes.

In the treatment groups, the SGOT level increased significantly ($p < 0.05$); however, the SGPT level increased insignificantly ($p > 0.05$). The increased SGOT activities indicated liver damages (Fig. 3), while the high levels of blood SGOT and SGPT attributed to the release of intracellular enzymes into blood vessels due to liver cell injury. FPBs have been reported to decrease SGOT and SGPT activities. For instance, soybean-based drinks exhibited a protective effect on the liver with acute injury induced by alcohol [30] and carbon tetrachloride (CCl_4) [31]. Chemically, soybeans contain isoflavones; with their potent antioxidant properties, they could repair tissue damages due to free radical species [32]. In addition, peptides derived from black soybean was reported of having alcalase, a protective agent against alcohol-induced liver damage [30], and arabinogalactan. Arabinogalactan, a class of polysaccharides composing of D-galactose and L-arabinose with hyperbranched chains, exerted a defense mechanism against CCl4-induced liver damage by suppressing increased SGOT and SGPT activities [31].

Based on the liver histopathology (Fig. 4), the FPB could repair the liver damage. The treatment groups showed a better histological profile than the negative control, suggesting the protective effects of FPB on a damaged liver due to lipid peroxidation created by free radicals.

Furthermore, this study found that excessive lipid accumulation caused by HFD led to high BUN and creatinine levels, statistically different from the normal and positive control groups ($p < 0.05$) (Fig. 2). The two main risk factors for chronic kidney disease (CKD) are obesity and hyperlipidemia, contributing to decreased renal function by lipid accumulation in the renal parenchyma [33]. Based on the results in this study, the FPB significantly reduced BUN and creatinine levels (Fig. 2). The reason could be the contribution of curcumin in *Z. cassumunar*, which contains anti-inflammatory and antioxidative properties,

to nephroprotection and slow kidney disease progression [34]. Moreover, the antioxidant components are responsible for the renoprotective effect [35].

Hyperlipidemia also contributes to the progress of kidney disease [33]. In this study, BUN and creatinine levels were significantly higher in the negative control than in the control group. This result corresponded to what revealed in a previous study that, in rats with a HFD, high BUN and creatinine levels were a response to kidney damage due to increased oxidative stress, strong inflammatory responses, and impaired glomerular filtration barrier [36].

In another study in rats and clinical studies in human, the safety of *Z. cassumunar* was reported. No detectable side effects (no-observed-adverse-effect-level) were found in rats orally receiving 1000 mg/kg/-day of *Z. cassumunar* powder for 90 days, and the consumption of *Z. cassumunar* tablets at a dose of 850 mg/man/day throughout four weeks of clinical research indicated that this dosage was safe for at least one month [37].

The European Medicinal Agency reported clinical evidence for ethanolic extracts of soya bean containing isoflavones to establish recognised efficacy and an acceptable level of safety [38]. The safety of *C. burmanii* extract was also reported that it did not cause any mortality nor any abnormalities in the necropsy and histopathology of treated rats. The LD_{50} for the *C. burmanii* extract was more than 2000 mg/kg, and no adverse effects were observed in rats treated with different doses of the extract [39].

CONCLUSION

The functional powdered beverage containing Cassumunar ginger (*Z. Cassumunar* Roxb.), soybean (*G. max*), and cinnamon (*C. burmannii*) showed protective effects against hyperlipidemia and injuries of liver, and kidney in rats given a high-fat diet.

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