Indonesian Journal of Pharmacy

VOL 32 (4) 2021: 529–538 | RESEARCH ARTICLE

Hepatoprotective Effect of Banana Peel Flour on Histological and Liver Function in Diabetic Rats

Herlin Ajeng Nurrahma[1]*, Andreanyta Meliala[2] Paramita Narwidina[3], Sri Herwiyanti[4]

1. Department of Physiology, Faculty of Medicine Universitas Sultan Agung Islamic, Jl. Raya Kaligawe Km.4 Semarang
2. Department of Physiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Farmako Street, Sekip Utara, Yogyakarta 55281, Indonesia
3. Clinical Nutrition Study Group, Yogyakarta, Indonesia Researcher in Clinical Nutrition Research Group, Yogyakarta 55133, Indonesia
4. Department of Histology and Cell Biology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Farmako Street, Sekip Utara, Yogyakarta 55281, Indonesia

Info Article

Submitted: 10-05-2021
Revised: 08-12-2021
Accepted: 18-12-2021

*Corresponding author
Herlin Ajeng Nurrahma

Email:
herlinajengn@gmail.com

ABSTRACT

Under long-term hyperglycemic conditions, metabolic abnormalities in the body can cause oxidative stress, with NAFLD being one of the complications. Banana peels are high in tryptophan, which is a precursor to serotonin in the body. Serotonin regulates body homeostasis, including blood glucose levels, and has also been linked to increased liver regeneration in rats. Based on the above, this study aimed to investigate the hepatoprotective effects of banana peel flour (BPF) on liver enzymes changes in rats. Twenty-five male Wistar rats were separated into five groups (n=5), including Group I was a healthy control group fed a standard diet, while Groups II to V were diabetic rats model groups that consumed a standard diet supplemented with BPF 0%, 5%, 10%, and 20%, respectively for four weeks. At the end of the experiment, the rats were bled and enzyme changes in ALT, AST, and Hematoxylin-Eosin (HE) staining were analyzed with the NAFLD score to examine hepatocellular morphology. The effect of BPF intervention on serum ALT and AST levels are not dosed dependent but the effects are comparable. BPF groups intervention produced a significant ($P<0.05$) decrease in serum levels of ALT and AST compared to the diabetic control group. The NAFLD score with HE staining showed substantial improvements in liver morphology, which was better seen at a 20% BPF dose. The current study supported the hypothesis that BPF had a hepatoprotective effect in diabetic rats and its effect may be due to the mechanism of controlling the hepatic enzyme transaminase and inducing liver regeneration.

Keywords: diabetes mellitus, NAFLD, BPF, liver function

INTRODUCTION

Type 2 diabetes mellitus (T2DM) in developing countries has increased dramatically which is often associated with sedentary lifestyle and obesity (Halter *et al*., 2014). In patients with DMT2, hyperglycemic conditions cause metabolic abnormalities in the body which if prolonged will have complications, one of which is a liver disease (Patel *et al.,* 2011; Tan *et al.,* 2015) such as Non-alcoholic fatty liver disease (NAFLD). NAFLD is a type of liver disease that occurs without the consumption of alcohol, which can be confirmed by laboratory microscopic and ultrastructural anatomy tests (Eren *et al*., 2003). The pathogenesis of NAFLD is not known with certainty, but already it has been strongly associated with metabolic syndrome and T2DM.

Indonesia is a tropical country that produces abundant bananas. In 2014, banana production in Indonesia reached 7,008,407 tons. The abundant production of bananas raises a classic problem: there is significant banana peel waste. In general, banana peels have not been used for any real purpose, except as disposable waste and rats feed. One way to use banana peels so that they can be used for a long time and are flexible in their use in

Copyright © 2021 by Indonesian Journal of Pharmacy (IJP). The open access articles are distributed under the terms and conditions of Creative Commons Attribution 2.0 Generic License (https://creativecommons.org/licenses/by/2.0/).

food products is by processing them into the form of flour. Remarkably, the nutritional content contained in BPF has benefits if it can be used as the right raw food material (Keszthelyi and Troost, 2009). The content in banana peels is not much different from the banana fruit itself. The mineral content in banana peels reaches 1,910 mg/kg and vitamin B6 content is 0.8 mg. Besides these essential nutrients, banana peels also contain the amino acid tryptophan as much as 2.5 mg/kg (Meliala *et al.,* 2020), also contain other ingredients such as pectin, lignin, cellulose, and hemicellulose which lower the blood sugar levels, as a result, will improve liver function (Lattimer & Haub, 2010)

BPF also contains tryptophan and its precursors, wherein tryptophan is a precursor to serotonin in the body (Rådholm *et al* 2016). Serotonin (serotonin) is a neurotransmitter that plays a key role in the Central Nervous System (CNS) and the autonomic nervous system (ANS), several studies have been conducted to determine the role of serotonin in regulating body homeostasis through the ANS, with blood glucose levels being one of them (Huskisson *et al.,* 2007), serotonin is involved in the mechanism of hepatic glucose uptake and glycogen metabolism, which helps to support liver cell regeneration and maintain glucose and insulin homeostasis (Lesurtel *et al*., 2012). The ability of serotonin to regulate hepatic glycolysis via the phosphofructokinase (PFK) activity regulatory pathway (Coelho *et al*., 2012)

Due to their natural tendency to be damaged quickly after harvest, banana peels must be processed into more durable food products with low water content, one of which is flour. BPF is more adaptable and can be found in a variety of diabetic-friendly processed foods. Few studies have investigated the link between BPF dietary quality and liver function parameters, particularly when tryptophan, a serotonin precursor, is present. Based on this, the purpose of this study was to analyze whether BPF has hepatoprotective properties supported by the various phytochemical compositions contained in it.

MATERIAL AND METHODS

All of the chemicals used in the experiments were analytical grade, as follows: Streptozotocin (STZ)and Anti-HTR2B antibody produced in rabbits were purchased from Sigma Chemicals Co, St Louis, MO, USA. A fine test rabbit, DAB detection kit, was purchased from Wuhan Fine Biotech, China. Materials for hematological research, including Hematoxylin, Eosin, and Victoria blue were used to stain the cells. Liver function evaluation was analyzed by measuring AST and ALT levels in the serum by using Diasys reagents via spectrophotometry method.

Preparation of BPF.

Yellow Kepok bananas (*Musa balbisiana* Colla) in stage 5 of ripening characterized by yellow skin with the green tip (Aurore *et al.,* 2009) were used in this study. The bananas were obtained from traditional markets in Sleman, Yogyakarta, Indonesia. Banana peel washed under running water, sliced into small pieces, soaked in 0.5% citric acid solution, steamed for 5 minutes, and mashed with a blender. The following is the ratio of banana peel: water = 1: 2 for 3 minutes, then dried in a drum dryer at a pressure of 3-4 bar, at a temperature of 140^{o}C, then mashed in a disk mill to make flour particles that move through an 80 mesh sieve.

Chemical composition analysis of experimental diets

Proximate composition of the samples including moisture (Method 925.40), ash (Method 950.49), protein (Method 955.04), fat (Method 920.39), and crude fiber (Method 935.53) was determined according to the Association of Analytical Chemist (AOAC) methods (AOAC, 2010). The serotonin and its precursor 5-Hydroxytryptophan (serotoninP) of experimental diet was analyzed via the reversed-phase HPLC method (Ly *et al*., 2008)

Ethical clearance

The present method was submitted to the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. The certificate of ethical clearance was numbered Ref: KE/FK/0313/EC/2020

BPF treatment

Twenty-five healthy adult male Wistar rats (*Rattus norvegicus*) weighing 150-220 g were used in this study. The rats were kept in laboratory rats housing, each in their polypropylene cage at the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Gadjah Mada following the recommendation of the Guide for the Care and Use of Laboratory Rats with constant 12 h light and dark cycle (08:00 AM – 08:00 PM), controlled temperature (22±2^{0}C) and humidity

(55±10%) with free access to cubes of standard diet food and tap water during the experiment. Before starting the experimental work, the rats were subjected to one week of acclimation period and various handling procedures to reduce the stress of novelty and handling.

The rats were classified into five groups with different diets for four weeks according to the methods described below: Group I (normal control, non-diabetic, administrated with diet standard AIN 93-M); Group II (diabetic control, diabetic, administrated with diet standard AIN-93 M); Group III (diabetic rats and administrated with standard diet containing 5% BPF), Group IV (diabetic rats and administrated with standard diet containing 10% BPF, and Group V (diabetic rats and administrated with standard diet containing 20% BPF). Diabetic rats model groups were injected intraperitoneally with STZ (60 mg/kg BW); Sigma-Aldrich, St Louis, MO) diluted in citrate-buffered saline (0.1 mol/l, pH 4.5; Sigma-Aldrich). After STZ induction, the rats had free access to 5% of dextrose for 72 h. A total of twenty rats with blood glucose levels of more than 250 mg/dL were included in this study.

The blood samples were collected from the orbital sinus under anesthesia using 50 mg/kg of ketamine, intramuscularly at day 0 (initial database) and 21 after 12h fasting. The blood was then allowed to stand at room temperature for 30 min. The samples were then centrifuged at 3600 rpm for 10 min to provide serum. The blood glucose analysis results were determined based on an enzymatic colorimetric method using glucose GOD-PAP (Ariastuti *et al.,* 2020). The hepatic enzyme transaminase ALT and AST) were examined with micro lab photometry. Hematoxylin-Eosin (HE) staining was analyzed to examine hepatocellular morphology with a NAFLD score.

Preparation of liver preparate

At the end of the experiment period, the rats were sacrificed under anesthesia, using 50 mg/kg of ketamine and after decapitation, liver tissue was collected, excised, and fixed in 10% buffered formalin at room temperature for no longer in 24h.

Delipidation

All liver tissues were dehydrated with graded ethanol 50%, 70%, 80%, 90%, 95%, and absolute, for 5 min at each concentration. Next, the tissues were soaked in pure xylene until no changed color and then rehydrated with absolute ethanol, 95% ethanol, 75% ethanol, and distilled water for 1 h, 0.5 h, 1 h, and 1 h at 60-70°C.

Hematoxylin-Eosin (HE) staining

The tissues were first stained for 6 h at 60–70°C with Harris' hematoxylin solution, then rinsed in tap water until the water was colorless. Following that, the tissue was differentiated twice with 10% acetic acid and 85% ethanol in water for 2 h and 10 h, respectively, before being rinsed with tap water. In the bluing step, the tissue was soaked in a saturated lithium carbonate solution for 12 h and then rinsing it with tap water. Finally, eosin Y ethanol solution was used to stain the cells for 48 h.

Paraffin embedding

The tissues were dehydrated twice for 0.5 h in 95 % ethanol, then soaked in xylene for 1 h at 60–70°C, followed by paraffin for 24 h.

Slicing and imaging

The stained tissues were cut into 4 μm in width using a microtome, dewaxed, mounted with DPX Mountant (Merck KgaA, Darmstadt, Germany), and then imaged using Olympus CX21/Optilens Optilab Standard light microscope (Carl Zeiss, Oberkochen, Germany) and photographed with a digital camera

Assessment of NAFLD

After the HE staining was completed, an assessment of the histology of the liver was done using the histological scoring system for NAFLD which was validated by the pathology committee of the NASH Clinical Research Network. The liver was scored for the presence of steatosis, ballooning, and lobular inflammation. Steatosis was graded 0–3 based on the percentage of hepatocytes in the biopsy involved with grades 0, 1, 2, and 3 showing involvement of < 5%, 5–33%, 33–66%, and > 66% hepatocytes, respectively. The severity of hepatocyte ballooning was graded from 0 to 2 with grades 0, 1, and 2 showing none, scattered and marked ballooning, respectively. Lobular inflammation was graded 0–3 based on inflammatory foci with grades 1, 2, and 3 showing < 2, 2–4, and > 4 inflammatory foci, respectively. (Kleiner *et al*., 2005)

Table I. Chemical composition of the experimental diets

Samples	Standard diet	Chemical composition (%)		
		Standard diet containing		
		5% BPF	10% BPF	20% BPF
Moisture	10.33±0.07[a]	10.25±0.16[a]	11.50±0.16[b]	14.38±0.17[c]
Ash	3.52±0.13[a]	3.78±0.16[ab]	3.97±0.18[bc]	4.61±0.04[c]
Protein	10.36±0.03[a]	10.28±0.04[a]	9.97±0.03[b]	8.39±0.07[c]
Fat	2.36±0.09[a]	2.98±0.08[ab]	3.67±0.37[bc]	4.16±0.4[c]
Crude fiber	4.54±0.05[a]	6.61±0.99[b]	9.11±0.09[c]	11.20±0.01[d]
Carbohydrate	68.89±0.10[a]	66.11±0.30[b]	61.79±0.05[c]	57.25±0.16[d]
5-HTP	ND	ND	0.06±0.01[a]	0.58±0.07[a]
Serotonin	0.02±0.00[a]	0.19±0.01[b]	1.39±0.55[c]	3.84±0.21[d]

The values represent the mean±SD. ND, not detected. Different superscripts in the same row show significant differences ($p \leq 0.05$). The ANOVA test was used and continued with the LSD test on all parameters.nd. 5-HTP: 5-Hydroxy Tryptophan

Statistical analysis

Data are expressed as mean ± S.D. of 5 rats in each group and statistically analyzed using The One-way ANOVA was followed by Least Significant Difference (LSD) post-hoc test (SPSS 23 for Windows, IBM Corp., Armonk, NY). The P-values <0.05 were used as the criterion for determining the level of significance. For assessment of NAFLD, the data were also evaluated using kappa statistics, which measure agreement. The Kappa estimates were interpreted according to Landis and Koch's guidelines for κ≤0, poor agreement; κ>0 but ≤0.20, slight agreement; κ>0.20 but ≤0.40, fair agreement; κ>0.40 but ≤0.60, moderate agreement; κ>0.60 but ≤0.80, substantial agreement; and κ>0.80 to 1.00, almost perfect agreement (Batistatou *et al*., 2013). In this study, we found moderate agreement (Kappa coefficient 0.789) between expertise in pathological anatomy and observer, so the bias that occurs could be minimized.

RESULTS AND DISCUSSION

Chemical composition of experimental diet

The proximate composition of the standard diet and standard diet containing 5%,10%, and 20% BPF which includes moisture, ash, fat, protein, crude fiber, and carbohydrate (Table I). All of the data was collected on a wet basis and expressed as a percentage of the total. The experimental diets with 5%, 10%, and 20% of BPF led to increasing the percentage of crude fiber and carbohydrate while the percentage of protein and fat were decreased. Statistical analysis by ANOVA and LSD test showed some significant differences between the adding of BPF. Increased percentage of carbohydrate content with the addition of BPF is related to the increased fibers and moisture content. The results of proximate composition of a standard diet containing 20% BPF have given the best nutritional value (crude fiber, carbohydrate, ash). Statistical analysis showed some significant differences between the diets ($p<0.05$). An increased percentage of BPF content is related to the increased serotonin content.

Effect on rat's liver function

The changes in AST and ALT levels by BPF intervention in the diet. There was a significant decrease ($p = 0.022$ and (Figure 1) $p = 0.005$) in both a standard diet containing 5% and 20% BPF groups compared to the diabetic control group and intervention with BPF 20% appeared to significantly ($p = 0.026$) reduce the serum level of AST compared to the normal control group. Our study also showed that the effect of the 5% BPF intervention was not significantly different when compared to the 10% BPF ($p = 0.501$) and 20% BPF ($P=0.177$), and the 10% BPF did not show a significant difference ($p = 0.070$) when compared with the 20% BPF diet intervention group.

The levels of ALT in the serum of the standard diet containing 5%, 10%, and 20% groups showed a significant decrease ($P=0.000$) compared to the diabetic control group (Figure 2). The interesting thing that we found in this study was that the decrease in serum ALT levels in the 5% BPF intervention was not significantly different ($p = 0.594$) compare to the group given the 10% BPF and when compared to the 5% BPF dietary intervention, the decrease in the 20% BPF dietary intervention was more significant ($p = 0.003$).

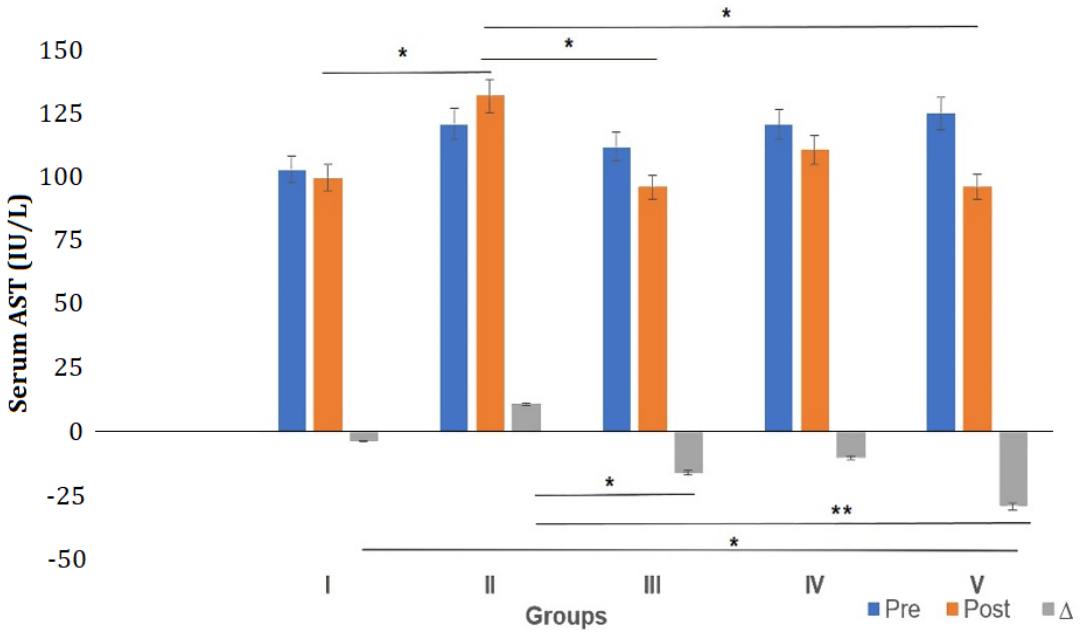

Figure 1. Serum levels of AST in diabetic rats fed on BPF supplemented diet. Group I: normal control, Group II: Diabetic control, Group III: Diabetic+BPF 5%, Group IV: Diabetic+BPF 10%, Group V: Diabetic+BPF 20%. Data were presented as mean±SD, n=5.
[a]P<0.05 vs. Group I, [b]P<0.05 vs Group 2. according to the one-way ANOVA followed by the LSD post-hoc test. AST, aspartate aminotransferase; BPF, banana peel flour

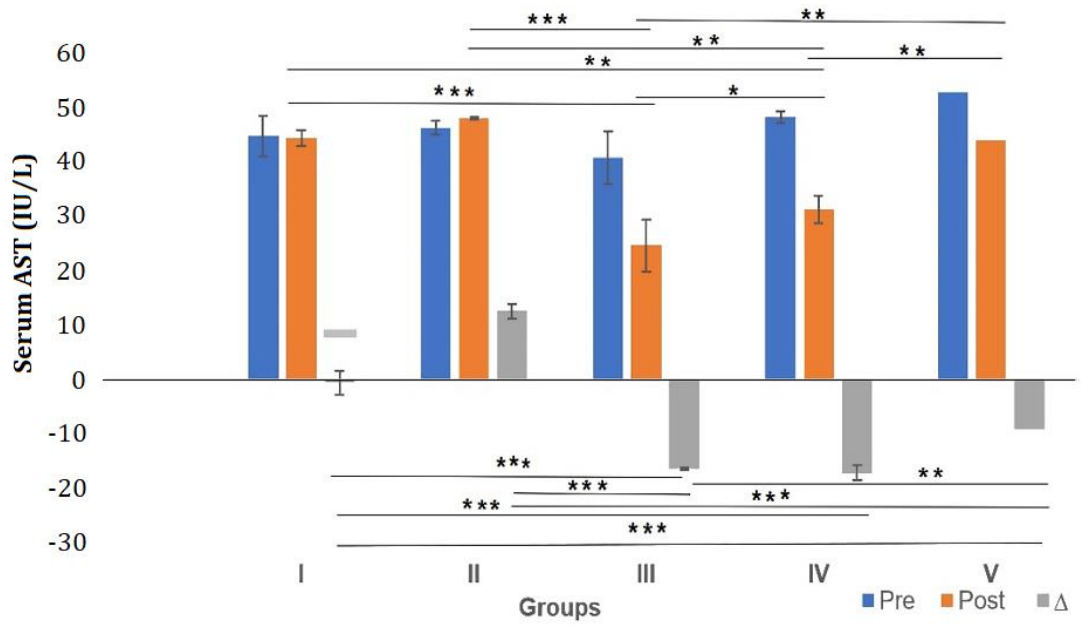

Figure 2 Serum levels of ALT in diabetic rats fed on BPF supplemented diet. Group I: normal control, Group II: Diabetic control, Group III: Diabetic+BPF 5%, Group IV: Diabetic+BPF 10%, Group V: Diabetic+BPF 20%. Data were presented as mean±SD, n=5.
[a]P<0.05 vs. Group I, [b]P<0.05 vs Group 2, [c]P<0.05 vs Group 3, [d]P<0.05 vs Group 4, [e]P<0.05 vs Group 5 according to the one-way ANOVA followed by the LSD post-hoc test. AST, aspartate aminotransferase; BPF, banana peel flour

This result confirmed that AST serum was alleviated by hepatoprotective activities from BPF, but it was not dose-dependent. The results were following previous reports (Famii & Ebuka, 2019), but different from those of other studies, which found that groups given a 50 percent ethanolic whole plant extract of Musa paradisiaca in doses of (200 and 400 mg/kg) once daily for 14 days were able to avoid hepatotoxicity in a dose-dependent manner (Sultana *et al*., 2012). In this study, the simple hepatic function can be seen from the levels of the transaminase enzyme. AST is an enzyme produced in mitochondria found in the liver, heart, kidneys, and brain. This enzyme plays a role in converting aspartate and α-ketoglutarate to oxaloacetate and glutamate (Wurtman *et al.,* 2003).

These results indicate that the effect of BPF intervention on serum ALT and AST levels are not dose-dependent but the effects are comparable. All the groups BPF intervention produced a significant decrease in serum of ALT when compared to the diabetic control group. Therefore, the results of the present study indicated that BPF can significantly reduce the levels of several key markers of hepatic injury, and these effects were comparable to the normal control rats group. ALT is the main enzyme found in liver cells that serves to catalyze the transfer of amino acids from alanine to α-ketoglutarate. The leakage of transaminase enzyme from the cells causes an increase in transaminase enzyme in serum (Wurtman *et al.,* 2003). The level of enzyme ALT exhibited a significant decrease in the experimental groups with BPF-fed diet compared to the diabetic control group ($p < 0.05$) (Figure 2). The results were following previous reports and the hepatoprotective potential of BPF is possible as a result of its antioxidant properties

Mitochondrial damage can also occur due to oxidative stress and reactive oxygen species (ROS) that are produced in large quantities in a hyperglycemic state, resulting in an increase in AST levels due to the intracellular release of enzymes into the blood, however, ALT is the main enzyme found in liver cells. This enzyme is also found in small amounts in muscles, the heart, kidneys, and skeletal muscles. The increase in the ALT is more prominent when there is partial damage to liver cells mainly in the membranes of the liver cells. The hyperglycemic state is related to insulin resistance in every cell in the body, one of which is the liver. Insulin resistance affects the permeability of the cell membrane so that the change in permeability and accumulation of ROS causes the ALT enzyme in the cytoplasm of the liver cells to leak and there will be an increase in AST levels in the bloodstream. In this condition, the liver is adversely affected by fat deposition, inflammation, with or without fibrosis (Salvatore *et al*., 2008). Banana peel with various nutritional compounds caused a decrease of the AST enzyme which was seen from a significant difference in the AST parameter. This is because Kepok BPF contains vitamins, minerals, proteins, and antioxidants that can improve enzyme regulation in the liver (Meliala *et al*., 2020; Mosa & Khalil, 2015). Antioxidants are molecules that can prevent other molecules from oxidizing. Free radicals are produced during oxidation reactions, which can harm cells. Antioxidants can neutralize free radicals. The antioxidants contained in Kepok BPF are vitamin C, pectin, saponins, and tannins, where tannins are the largest content, reaching 1,236 mg per 100 grams of BPF (Meliala *et al*., 2020).

In addition, BPF also has a high content of tryptophan dan serotonin, where serotonin has been known to have various biological functions in liver cell metabolism. One of them is the effect of serotonin in the Hepatic Stellate Cell (HSC) which has an important role in the response to liver damage (Ruddell *et al.,* 2008). Serotonin has a role in HSC proliferation and inhibition of apoptosis of hepatocytes, but other studies suggest that serotonin can also increase the apoptotic process of hepatocytes and prevent the proliferation of HSCs (Antonio *et al*., 2007). The group of diabetic rats fed with variation of Kepok BPF had the impact reducing AST and ALT levels.

Effect of BPF on the histopathology of the liver

The NASH Clinical Research Network's pathology committee has approved a histological scoring system for NAFLD, which looked for steatosis, lobular inflammation, and ballooning degeneration to see if there was any hepatocellular damage in liver cells stained with HE. The mean results of observations of rat liver cells with HE staining on all parameters, namely steatosis, lobular inflammation, ballooning degeneration, and the total score (Table II) and the representative images of the histopathological observation of the liver of rats at the different groups Figure 3). Rats in the normal control group showed normal architecture of the liver with healthy hepatocytes and central vein. Liver from the rats in the BPF fed diet groups showed severe fatty deposition, ballooning, and lobular inflammation.

Table II. Mean NAFLD scores in rat liver cells stained with Hematoxylin-Eosin

Group	Mean NAFLD scores in rat liver cells			
	Steatosis	**Lobular inflammation**	**Degeneration Ballooning**	**Total Score**
I	0.32±0.20[a]	0.76±0.97[a]	0.28±0.13[a]	1.36±0.24[a]
II	2.12±0.32[b]	1.64±0.11[b]	1.64±0.12[b]	5.40±0.46[b]
III	1.24±0.29[ac]	1.28±0.18[bc]	1.40±0.14[c]	3.76±0.44[c]
IV	1.00±0.22[c]	1.12±0.14[ac]	0.92±0.13[cd]	3.20±0.41[d]
V	0.68±0.08[ac]	0.84±0.14[a]	0.76±0.11[ac]	2.28±0.30[e]

The values represent the mean±SD. Group I: normal control, Group II: Diabetic control, Group III: Diabetic+BPF 5%, Group IV: Diabetic+BPF 10%, Group V: Diabetic+BPF 20%. Different superscripts in the same column show significant differences ($P < 0.05$). The One Way ANOVA Test was used followed by the least significant difference (LSD) test on all parameters.

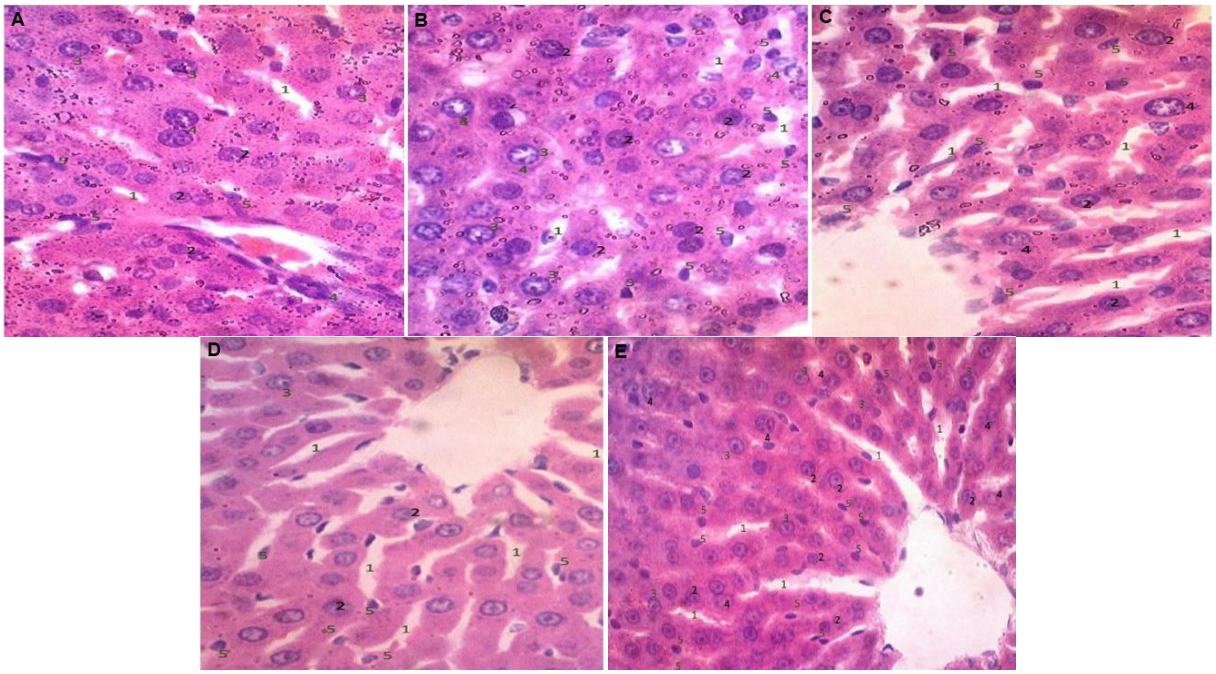

Figure 3. Microscopic image of mouse liver cells with Hematoxylin-Eosin staining at 400x magnification. (A) The liver cells in the control group are normal; (B) Picture of hepatic cells in the Diabetic control group; (C) Picture of hepatic cells in the Diabetic + BPF group 5%; (D) The picture of hepatic cells in the Diabetic + BPF group is 10%; (E) Hepatic cells in the Diabetic + BPF group 20%. (1). sinusoids, (2) normal hepatocytes, (3) steatosis, (4) degeneration ballooning, and (5) lobular inflammation

As a result, the One Way Anova test was conducted, followed by LSD's post-hoc analysis. There were significant ($p < 0.05$) differences between groups of BPF intervention in steatosis, lobular inflammation, ballooning degeneration, and total parameters. In the steatosis parameter, diabetic rats given BPF 5%, 10%, and 20% showed significant ($p = 0.004$, $p = 0.018$, and $p = 0.000$) reduction in liver steatosis compared to diabetic rats with a standard diet.

The liver of rats with BPF 10% and 20% intervention showed significant ($p = 0.016$ and $p = 0.038$) lobular inflammation compared to the diabetic control, but an insignificant result ($p = 0.083$) was seen in the liver of rats treated with BPF 5%. Table 2 also shows that the presence of stratified doses of BPF had no significant impact on the appearance of lobular inflammation, where the liver group with 5% and 20% BPF intervention was not significantly different ($p = 0.428$ and $p = 0.172$)

compared to the liver condition in the group with 10% BPF intervention. This shows the effect of BPF on the occurrence of lobular inflammation, not dose-dependent. There were significant differences between groups in terms of ballooning degeneration parameters.

The presence of diabetes conditions caused the degeneration view to balloon, indicating a significant difference from normal conditions and the group that consumed BPF at doses of 5% ($p = 0.002$), 10% ($p = 0.000$), and 20% ($p = 0.016$). Muti-dose of BPF administration did not have a significant impact on the appearance of ballooning degeneration, where the liver group with 10% and 20% BPF intervention did not show a significant difference ($p = 0.205$ and $p = 0.393$) compared to the liver condition in the group with BPF 5% intervention. This shows that the effect of BPF on the microscopic feature of ballooning degeneration is not dose-dependent.

The total score parameter showed a significant difference between groups, with the normal rat group having the lowest score, followed by the treatment groups, which consisted of diabetic rats treated with a standard diet containing 5%, 10%, and 20% of BPF, and then the diabetic rats' group with standard feed which had the highest overall score, but the effect caused by the 5% BPF intervention was not significantly different from the 10% and 20% BPF intervention with p-value = 0.212 and 0.104. Hyperglycemia causes organs in the body, including the liver, to lose their balance in the diabetic condition. The liver will increase glucose uptake, resulting in biochemical and morphological changes in the organ.

Observations in the liver cell with HE staining were assessed using a histological scoring system for NAFLD, which was observed for steatosis, lobular inflammation, and ballooning degeneration to determine whether there was any hepatocellular damage. The following is a sample of the HE staining from each group (Figure 1). Changes in the liver had begun to occur in the hyperglycemic state, as evidenced by an increase in the ALT and AST enzymes, as well as structural changes in the liver. The mitochondria, rough endoplasmic reticulum, and nucleus of hepatocytes showed the most ultrastructural variation. However, whether these structural changes are caused by pure hyperglycemia or by STZ and alloxan, which are used to cause experimental liver damage in rats, is still unknown. (Luchchesi *et al.,* 2013). Damage to the hepatic cell structure is closely related to duration, with damage beginning to appear at week 2 and progressing to week 26 in experimental rats with uncontrolled hyperglycemia conditions (Bilal *et al.,* 2017).

Due to the presence of its phenolic hydroxyl group, serotonin in foods acts as an antioxidant compound in the mechanism of radical scavenging activity, according to various research findings (Gulcin, 2008; Sarikaya & Gulcin, 2013). The consumption of foods containing serotonin as an antioxidant can boost their physiologic concentrations in the blood, boosting antioxidant defenses, improving mood, and treating sleep disorders, depression, and anxiety (Gonçalves *et al*., 2021), serotonin is also the precursor of melatonin, this one is capable to reduce fibrosis, inflammation, and liver tissue injury in rats (Colares *et al*., 2016).

CONCLUSIONS

The intervention of BPF with several variations in diabetic rats caused a significant improvement in the morphology of cells in the liver, according to this study. This could be due to the presence of its phenolic hydroxyl group from serotonin acts as an antioxidant compound in the mechanism of radical scavenging activity high content of antioxidant active substances in BPF, as well as from serotonin, allowing for a good regeneration process in the liver cells. Based on these results, BPF provided a hepatoprotective effect in the diabetic rat model, which might occur through the mechanism of controlling gluconeogenesis and liver regeneration.

ACKNOWLEDGEMENTS

We thank the Directorate of Research Universitas Gadjah Mada, through Rekognisi Tugas Akhir (RTA) scheme Fiscal year 2020

REFERENCES

Antonio, N., Dahm, F., Jochum, W., Jang, J. H., Georgiev, P., Bader, M., Renner, E. L., & Clavien, P.-A. (2007). Serotonin mediates oxidative stress and mitochondrial toxicity in a murine model of nonalcoholic steatohepatitis. *Gastroenterology*, *133*(2), 608–618. https://doi.org/10.1053/J.GASTRO.2007.05.019

AOAC. (2010). *Official Methods of Analysis.Association of Official Analytical Chemists* (18th ed.).

Ariastuti, R., Fitrawan, L. O. M., Nugroho, A. E., & Pramono, S. (2020). Antidiabetes of

combination of fractionated-extracts of andrographis paniculata and centella asiatica in neonatal streptozotocin-induced diabetic rats. *Indonesian Journal of Pharmacy*, *31*(4), 312–322. https://doi.org/10.22146/ijp.1135

Aurore, G., Parfait, B. and Fahrasmane. (2009). Bananas, raw materials for making processed food products. *Trends in Food Science and Technology*, *20*, 78–91.

Batistatou, A., Televantou, D., Bobos, M., Eleftheraki, A. G., Kouvaras, E., Chrisafi, S., Koukoulis, G. K., Malamou-Mitsi, V., & Fountzilas, G. (2013). Evaluation of Current Prognostic and Predictive Markers in Breast Cancer: A Validation Study of Tissue Microarrays. *ANTICANCER RESEARCH*, *33*, 2139–2146.

Bilal, H. M., Riaz, F., Munir, K., Saqib, A., & Sarwar, M. R. (2017). Histological changes in the liver of diabetic rats : A review of pathogenesis of nonalcoholic fatty liver disease in type 1 diabetes mellitus. *Cogent Medicine*, *4*(December). https://doi.org/10.1080/2331205X.2016.1275415

Coelho, W. S., Da Silva, D., Marinho-Carvalho, M. M., & Sola-Penna, M. (2012). Serotonin modulates hepatic 6-phosphofructo-1-kinase in an insulin synergistic manner. *The International Journal of Biochemistry & Cell Biology*, *44*(1), 150–157. https://doi.org/10.1016/J.BIOCEL.2011.10.010

Colares, J. R., Schemitt, E. G., Hartmann, R. M., Licks, F., Soares, M. do C., Bosco, A. D., & Marroni, N. P. (2016). Antioxidant and anti-inflammatory action of melatonin in an experimental model of secondary biliary cirrhosis induced by bile duct ligation. *World Journal of Gastroenterology*, *22*(40), 8918. https://doi.org/10.3748/WJG.V22.I40.8918

Eren, I., Erdi, O., & Ozcankaya, R. (2003). Relationship between blood glucose control and psychiatric disorders in type II diabetic patients. *Türk Psikiyatri Dergisi*, *14*(3), 184–191.

Famii, Z. L., & Ebuka, U. C. (2019). Hepatoprotective activities of aqueous extract of oyster mushroom (Pleurotus ostreatus) against lead-induced hepatotoxicity in albino Wistar rats (Rattus norvegicus). *World Journal of Advanced Research and Reviews*, *03*(03), 33–37. https://doi.org/10.30574/wjarr

Gonçalves, A. C., Nunes, A. R., Alves, G., & Silva, L. R. (2021). Serotonin and Melatonin: Plant Sources, Analytical Methods, and Human Health Benefits. *Revista Brasileira de Farmacognosia*, *31*(2), 162–175. https://doi.org/10.1007/s43450-021-00141-w

Gulcin, I. (2008). Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent Ilhami Gülçin Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *23*(6), 871–876. https://doi.org/10.1080/14756360701626223

Halter, J. B., Musi, N., Horne, F. M. F., Crandall, J. P., Goldberg, A., Harkless, L., Hazzard, W. R., Huang, E. S., Kirkman, M. S., Plutzky, J., Schmader, K. E., Zieman, S., & High, K. P. (2014). Diabetes and cardiovascular disease in older adults: Current status and future directions. In *Diabetes* (Vol. 63, Issue 8, pp. 2578–2589). American Diabetes Association Inc. https://doi.org/10.2337/db14-0020

Huskisson, E., Maggini, S., & Ruf, M. (2007). The role of vitamins and minerals in energy metabolism and well-being. In *Journal of International Medical Research* (Vol. 35, Issue 3, pp. 277–289). Cambridge Medical Publications. https://doi.org/10.1177/147323000703500301

Keszthelyi D, Troost FJ, M. A. (2009). Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. *Neurogastroenterology & Motility*, *21*(12), 1239–1249.

Kleiner, D. E., Brunt, E. M., Natta, M. Van, Behling, C., Contos, M. J., Cummings, O. W., Ferrell, L. D., Liu, Y.-C., Torbenson, M. S., Unalp-Arida, A., Yeh, M., Mccullough, A. J., & Sanyal, A. J. (2005). Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease. *Hepatology*, *41*(6), 1313–1321. https://doi.org/10.1002/hep.20701

Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients*, *2*(12), 1266–1289. https://doi.org/10.3390/nu2121266

Lesurtel, M., Soll, C., Humar, B., & Clavien, P. (2012).

Serotonin : A double-edged sword for the liver ? *Surgeon*, *10*(2), 107–113. https://doi.org/10.1016/j.surge.2011.11.002

Luchchesi, N. A., Freitas, T. N., Cassetari, L. L., Marques, F. G. S., & Spadella, T. C. (2013). Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: a mechanism for diabetic chronic liver disease. *Acta Cirurgica Brasileira*, *28*(7), 502–508.

Ly, D., Kang, K., Choi, J.-Y., Ishihara, A., Back, K., & Lee, S.-G. (2008). HPLC Analysis of Serotonin, Tryptamine, Tyramine, and the Hydroxycinnamic Acid Amides of Serotonin and Tyramine in Food Vegetables. *Journal of Medicinal Food*, *11*(2), 383–389. https://doi.org/10.1089/jmf.2007.514

Meliala, A., Andwi, Y., Sumiwi, A. R. I., Narwidina, P., Sulistyo, S. R. I. L., & Setyaningsih, W. (2020). Banana Peel Flakes Alleviate Blood Glucose And Stress In A Dose-Dependent Manner. *International Journal of Pharmacy and Pharmaceutical Sciences*, *12*(8), 75–81. https://doi.org/https://doi.org/10.22159/ijpps.2020v12i8.37659

Mosa, Z. M., & Khalil, A. F. (2015). The effect of banana peels supplemented diet on acute liver failure rats. *Annals of Agricultural Sciences*, *60*(2), 373–379. https://doi.org/10.1016/j.aoas.2015.11.003

Patel, D. K., Kumar, R., Prasad, S. K., Sairam, K., & Hemalatha, S. (2011). Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, *1*(4), 316–322. https://doi.org/10.1016/S2221-1691(11)60051-8

Rådholm, K., Wiréhn, A. B., Chalmers, J., & Östgren, C. J. (2016). Use of antidiabetic and antidepressant drugs is associated with increased risk of myocardial infarction: A nationwide register study. *Diabetic Medicine*, *33*(2), 218–223. https://doi.org/10.1111/dme.12822

Salvatore P, Cammà, C., Marco, V. Di, Alessi, N., Cabibi, D., Caldarella, R., Licata, A., Massenti, F., Tarantino, G., Marchesini, G., & Craxì, A. (2008). Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. *The American Journal of Gastroenterology*, *103*(5), 1136–1144. https://doi.org/10.1111/J.1572-0241.2008.01813.X

Sarikaya, S., & Gulcin, I. (2013). Radical Scavenging and Antioxidant Capacity of Serotonin. *Current Bioactive Compounds*, *9*(2), 143–152. https://doi.org/10.2174/22115528112019990006

Sultana, M., Verma, P., Raina, R., Prawez, S., & Dar, M. (2012). Quantitative Analysis of Total Phenolic, Flavonoids and Tannin Contents in Acetone and n-hexane Extracts of Ageratum conyzoides. *Int. J. Chemtech Res.*, *4*.

Tan, K. C., Chan, G. C., Eric, H., Maria, A. B. I., Norliza, B. M. J., Oun, B. H., Sheerine, A. B. M. T., Wong, S. J., & Liew, S. M. (2015). Depression, anxiety and stress among patients with diabetes in primary care: A cross-sectional study. *Malaysian Family Physician*, *10*(2), 9–21. /pmc/articles/PMC4826577/

Wurtman, R. J., Wurtman, J. J., Regan, M. M., McDermott, J. M., Tsay, R. H., & Breu, J. J. (2003). Effects of normal meals rich in carbohydrates or proteins on plasma tryptophan and tyrosine ratios. *American Journal of Clinical Nutrition*, *77*(1), 128–132. https://doi.org/10.1093/ajcn/77.1.128