Amerta Nutrition
e-ISSN: 2580-1163 (Online)
p-ISSN: 2580-9776 (Print)
Mulyadewi et al. | Amerta Nutrition Vol. 8 Issue 3SP (Desember 2024). 412-421

RESEARCH STUDY
English Version
OPEN ACCESS

Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats

Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes

Amelia Luthfiyah Mulyadewi[1], Ibnu Malkan Bakhrul Ilmi[1*], Angga Hardiansyah[2]

[1]Program Studi S-1 Ilmu Gizi, Fakultas Kesehatan, Universitas Pembangunan Nasional "Veteran" Jakarta, Jalan RS. Fatmawati Raya, Pd. Labu, Kota Jakarta Selatan, Daerah Khusus Ibukota Jakarta, 12450, INDONESIA
[2]Program Studi Gizi, Fakultas Psikologi Kesehatan, UIN Walisongo, Jl. Prof. Dr. Hamka, Kota Semarang, Jawa Tengah, 50185, INDONESIA

ARTICLE INFO

Received: *17-09-2024*
Accepted: *31-12-2024*
Published online: *31-12-2024*

Correspondent:
Ibnu Malkan Bakhrul Ilmi
ibnuilmi@upnvj.ac.id

DOI:
10.20473/amnt.v8i3SP.2024.412-421

Available online at:
https://e-journal.unair.ac.id/AMNT

Keywords:
Diabetic, Kefir, Honey, Renal, Pancreas

ABSTRACT

Background: The prevalence of diabetes is rising. Chronic metabolic disease type 2 diabetes may damage renal function and harm the pancreas. One of the initiatives to prevent and manage type 2 diabetes through functional foods. The antidiabetic and antioxidant properties of goat milk kefir with honey are thought to help heal kidney and pancreatic damage.
Objectives: Evaluating the effects of feading fermented goat milk with honey on pancreatic histopathology and renal function in diabetic rats.
Methods: A true experimental study conducted on 42 male Sprague-Dawley rats aged 6-8 weeks were divided into Six groups: Healthy Rats (KS); DM Rats (KN); quercetin (K1); metformin (K2); Kefir (P1); Preventive (P2). Diabetic rats type 2 were conditioned by HFD and injecting streptozotocin. Oral kefir was given 1.8 ml/200g BW/day for 21 days to groups KK and KP. Renal function was analyzed with pre-post tests through measurement of serum levels urea and creatinine, while the histopathological features were evaluated at the end of the study. The Kruskal-Wallis and post hoc Mann-Whitney test are used to analyze the data.
Results: P1 rats had Creatinine level of 0.57 ± 0.07 mg/dL; BUN 27.56 ± 6.22 mg/dL; Beta Cell Count 104.2; Island Area of Langerhans 170.26. The levels of BUN (p-value=0.083), creatinine (p-value=0.016), and Langerhans island area (p-value=0.026). Creatinine of rat P1 were significantly different from KN (p-value<0.05).
Conclusions: Goat milk kefir with honey in type 2 diabetic rats show beneficial effects on serum BUN, creatinine, and histopathological features of the pancreas as treated with metformin.

INTRODUCTION

Diabetes mellitus (DM) poses a significant global health burden. According to the International Diabetes Federation (IDF) 2021, 537 million people worldwide (10.5%) are currently living with diabetes. Indonesia ranks fifth globally in terms of diabetes prevalence, with 19.5 million individuals affected. Notably, 73.3% of these cases remain undiagnosed. Compared to the previous decade, diabetes cases in Indonesia have surged dramatically by 12.2 million. Type 2 diabetes mellitus (T2DM) constitutes the predominant form of the disease[1]. The 2023 Indonesian Basic Health Research revealed that 11.7% of the Indonesian population aged 15 years and older have diabetes based on blood sugar test results. Furthermore, based on doctor's diagnosis about 50% of them are type 2 diabetes[2]. Type 2 diabetes mellitus is characterized by hyperglycemia resulting from insulin resistance, pancreatic β-cell dysfunction, or a combination of both. In the case of insulin resistance, body cells fail to respond effectively to insulin, leading to hyperglycemia. This resistance increases hepatic glucose production while simultaneously decreasing glucose uptake in muscle, liver, and adipose tissue. Consequently, pancreatic β-cells are overexerted, ultimately leading to β-cell dysfunction[3]. Histopathological changes observed in T2DM include loss and hypertrophy of pancreatic islets[4].

The World Health Organization (WHO) emphasizes the progressive nature of diabetes, highlighting its potential to damage the liver, eyes, blood vessels, nerves, and kidneys. Poorly controlled diabetes significantly increases the risk of complications, including kidney failure[5]. This strong association underscores the detrimental impact of diabetes on renal function. Hyperglycemia damages blood vessel walls, promoting atherosclerosis and narrowing of blood vessels. This reduced blood flow compromises renal blood supply, disrupting glomerular filtration and consequently

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

impairing kidney function[6,7]. This functional decline is manifested by elevated blood urea nitrogen (BUN) and creatinine levels. These findings are further supported by histological alterations observed in the kidneys of animal models[8]. Clinical evidence suggests that consuming functional foods can effectively prevent and delay the onset and progression of T2DM and its complications. These foods exert beneficial effects by improving glycemic control, lowering blood pressure, enhancing antioxidant activity, modulating gut microbiota, and reducing the production of pro-inflammatory cytokines in patients with diabetes[9].

Kefir is a popular traditional fermented milk product derived from various animal milk sources, including cow, goat, camel, buffalo, or horse[10]. While cow's milk is commonly used for kefir production in Indonesia, goat's milk offers several advantages as an alternative. Notably, goat's milk exhibits lower allergenicity, improved digestibility, and higher levels of short- and medium-chain fatty acids compared to cow's milk[11,12]. One serving of goat milk kefir (200 ml) provides 7.18 grams of protein, 4.04 grams of fat, 456 mg of calcium, 4.96 grams of iron, and 0.26 micrograms of vitamin B12[13]. Research has demonstrated the antidiabetic properties of goat milk kefir, highlighting its potential to improve pancreatic cell function and provide protection[14,15]. Despite its benefits to the human body, the consumption of goat's milk is still limited due to the distinctive flavor of goat's milk, which can limit consumer acceptance[12,16]. Therefore, honey has been successfully incorporated as a natural sweetener to enhance the palatability of goat milk kefir products. Furthermore, adding honey to the goat kefir product not only improves flavor but also significantly enhances the antioxidant activity of goat milk kefir[17]. studies have shown that low-dose honey consumption in T2DM patients results in a lower glycemic response compared to glucose consumption[18]. Honey, as an antidiabetic agent, possesses the potential to mitigate the adverse effects of diabetic complications on various organs[19,20]. Hence, this study aimed to analyze the potential of honey-enriched goat milk kefir on renal function and pancreatic histopathology in a rat model of type 2 diabetes mellitus.

METHODS

Study Design

This was a true experimental study with a randomized controlled design. The study was conducted from July to November 2024 at the iRATco Laboratorium Group research facility. Ethical approval for animal experimentation was obtained from the Prima Indonesia University Health Research Ethics Committee (KEPK) (approval number 041/KEPK/UNPRI/VII2024, dated July 15, 2024).

Equipment and Materials

The equipment used in this study includes a food thermometer, analytical balance, beaker glass, 5 ml measuring cylinder, 15 ml tube, wooden stirrer, food strainer, hotplate, aluminum foil, freezer, feeding tube, rat cages, rat food and drink containers, and a spectrophotometer. The materials used in the study include goat milk, cotton-tree flower honey (Madu Randu), kefir grains, Streptozotocin (STZ) 40 mg, Metformin, Quercetin, standard rodent diet, and a high-fat diet with a fat content of 50%, each formulated by iRATco Laboratory based on AIN93 standards. The BUN and creatinine testing equipment includes a micropipette, microplate, pipette, and spectrophotometer. The materials used for BUN and creatinine testing include reagents, ureum kits, and creatinine kits. For pancreatic and kidney histopathology testing, equipment such as tissue processing tools, paraffin dispensers, cassettes, slides, cover glasses, microtomes, jars, incubators, micropipettes, microtubes, and slide boxes are used. Materials needed for pancreatic and kidney histopathology tests include graded alcohol (70%, 80%, 90%, 95%, 100%), xylene, distilled water, hematoxylin and eosin, and paraffin.

Kefir Preparation

Goat milk was pasteurized at 72°C for 15 seconds and subsequently cooled to room temperature (±27°C). According to the predetermined formulation, kefir grains and cotton-tree flower honey were added to the cooled goat milk. The mixture was then fermented at room temperature (±25-27°C) for 24 hours. Finally, kefir grains were separated from the fermented product through filtration for subsequent use in the experiment[13,17].

Hotplate

Tube

Beaker

Analytical Balance

Measuring Cylinder 5ml

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*


Food Thermometer


Container and Stirrer


Alumunium Foil

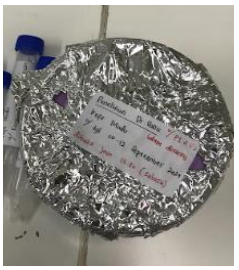

Goat Milk


Kefir Grains


Randu Honey (*Madu Randu*)


a. Pasteurization Process


b. Addition of Kefir Grains and Honey


c.Fermentation Process


d. Filtration Process of the Final Product

Figure 1. Equipment, Materials, and Process

Research Procedure

Male Sprague-Dawley rats, aged 6-8 weeks and weighing 120-150 grams, were obtained from iRatco Laboratory. A total of 42 rats were used in the study, divided into six groups (n=7 per group): a healthy control group (KS), a diabetic control group (KN), a diabetic group treated with quercetin (K1), a diabetic group treated with metformin (K2), a diabetic group treated with kefir (P1), and a preventive group (P2). This sample size was determined based on a recommended minimum of five rats per group, with an additional two animals included to account for potential dropouts[21,22]. Each group was housed in individually ventilated cages, with 3-4 rats per cage. The animal facility maintained a controlled environment with a temperature of 22 ± 3°C and a relative humidity of 50-60%. A 12-hour light/dark cycle was implemented. Standard rodent chow (18% crude protein, ≥3.5% fat, ≥5.5% crude fiber), formulated by iRATco Laboratory according to the AIN-93 guidelines, was provided ad libitum.

The experimental rats underwent a seven-day acclimatization period to adapt to the research environment[23]. The control groups (KN, K1, K2, and P1) were fed a high-fat diet containing 50% fat for four weeks. The preventive group (P2) received 1.8 ml of honey kefir per 200 g of body weight during this period. At the end of the fourth week, streptozotocin (STZ) at a dose of 50 mg/kg body weight was injected into the rat groups (KN, K1, K2, and P1) to create rats with Type 2 Diabetes Mellitus. T2DM was confirmed by monitoring blood glucose levels until they exceeded 200 mg/dL. Following diabetes induction, the following interventions were administered for 21 days: Group P1 and P2 received honey-kefir (1.8 ml/200 g body weight)[24,25]; group K1 received quercetin (15 mg/kg body weight)[25,26]; and group K2 received metformin (62.5 mg/kg body weight)[25]. The healthy control group (KS) received no intervention.

Kidney Function Assessment

Renal function was assessed by measuring blood urea nitrogen (BUN) serum and creatinine levels. Blood samples were collected from the retro-orbital sinus. The procedure for testing BUN levels begins with preparing tools (tube stand, 50 ml tube, serological pipette, stopwatch, spectrophotometer, vortex mixer, and microtube). The BUN assay was performed using a urea glory reagent kit (GD-UR100, Diagnostics). The working reagent was prepared by mixing 4 mL of R1 with 1 mL of R2. Subsequently, 1 mL of working reagent and 10 µL of sample/standard were added to a microtube. Absorbance was measured at 340 nm at 30 and 90 seconds using a spectrophotometer. Next, the creatinine assay was performed using a creatinine reagent kit (GD-CR100, Diagnostics). The procedure for testing creatinine levels in serum or plasma was performed by preparing tools (tube stand, 50 ml tube, serological pipette, stopwatch, spectrophotometer, vortex mixer, and microtube). The working reagent was prepared by mixing equal volumes of R1 and R2. Subsequently, 1 mL of working reagent and 10 µL of sample were added to a microtube. Absorbance was measured at 510 nm at 30 and 90 seconds using a spectrophotometer[27,28].

Histopathology of Pancreas and Kidney

Assessment of damage to kidney and pancreas

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

structures through histopathological analysis of organs begins with the trimming procedure. Tissue samples were fixed in 10% buffered neutral formalin (BNF). Subsequently, tissues were trimmed, embedded in cassettes, and dehydrated through a graded series of alcohol solutions (70%, 80%, 96%, 100%) for two hours each using a tissue processor. Following dehydration, tissues were cleared in xylene (three changes, 45 minutes each) and embedded in paraffin blocks. Sections (3-5 µm thick) were cut and stained with hematoxylin and eosin (H&E). The staining procedure involved deparaffinization in an incubator at 57°C for 15-30 minutes, followed by immersion in xylene solutions (I-IV) for 5 minutes each. Rehydration was performed by immersing the sections in graded alcohol solutions (100%, 95%, 90%, 85%, 80%, and 70%) for 5 minutes each, followed by a 5-minute wash in distilled water. Staining was conducted by immersing sections in hematoxylin for 5 minutes, followed by a 5-minute wash in running water, and then immersing in eosin solution for 5 minutes, followed by another 5-minute wash in distilled water. Subsequently, the sections were rehydrated through graded alcohol solutions (70%-100%), cleared in xylene (I-III) for 5 minutes each, and finally mounted with a coverslip. Kidney tissue damage was assessed through descriptive observations. Pancreatic β-cell number and islet area were measured using ImageJ software (NIH.gov) at 400x magnification[29].

Data analysis

Creatinine and BUN values, as well as the number and area of islets of Langerhans, were analyzed using analytical software. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. One-way ANOVA was employed to analyze differences in creatinine, BUN, and pancreatic β-cell number and area between groups. In cases where data did not meet the assumptions of normality or homogeneity, the Kruskal-Wallis test was used. Post-hoc comparisons were conducted using Duncan's test following one-way ANOVA or the Mann-Whitney U test following the Kruskal-Wallis test[30].

RESULTS AND DISCUSSIONS

Induction of Diabetes Mellitus in Experimental Rats

To stimulate metabolic syndrome in experimental rats, a high-fat diet was administered, followed by an injection of streptozotocin (STZ). After a 30-day conditioning period, rats with impaired renal function were successfully obtained, as evidenced by biochemical data shown in Table 1.

Table 1. Mean Values of BUN and Serum Creatinine Levels at Pre-Test

Kelompok	BUN (mg/dl)	Kreatinin (mg/dl)
KS	22.48 ± 2.75	0.69 ± 0.07
KN	27.56 ± 3.31	0.63 ± 0.11
K1	25.25 ± 6.08	0.59 ± 0.07
K2	21.00 ± 4.39	0.66 ± 0.09
P1	27.51 ± 7.46	0.69 ± 0.07
P2	20.44 ± 4.30	0.64 ± 0.03

The pre-test BUN levels across the KS, K1, K2, and P2 groups fell within the normal range, while the KN and P1 groups exhibited elevated mean values exceeding 26.4 mg/dL. The normal reference range for BUN in Sprague Dawley rats is 11.7–26.4 mg/dL, as reported in prior studies[31,32]. Statistical analysis using the Kruskal-Wallis test yielded a p-value of 0.069 (p-value > 0.05), indicating no significant differences in BUN values at pre-test among the six experimental groups. Similarly, the pre-test creatinine levels for all groups were within the normal range (0.5–0.83 mg/dL)[31,32]. Analysis of the pre-test creatinine levels revealed a p-value of 0.137 (p-value > 0.05), confirming the absence of significant differences between groups at the beginning of the study. At baseline, all 42 samples were complete. However, two rats died during the intervention period (accounted as dropout samples), reducing the sample size to 40. Chronic hyperglycemia, a hallmark of diabetes mellitus, often progresses to diabetic nephropathy, characterized by glomerular damage due to prolonged high blood glucose levels. Hyperglycemia induces the formation of advanced glycation end products (AGEs) and activates inflammatory pathways, leading to glomerular basement membrane thickening and impaired glomerular filtration rate (GFR). Consequently, renal dysfunction manifests as elevated blood levels of creatinine and urea[6,33].

BUN and Creatinine Levels After Intervention

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

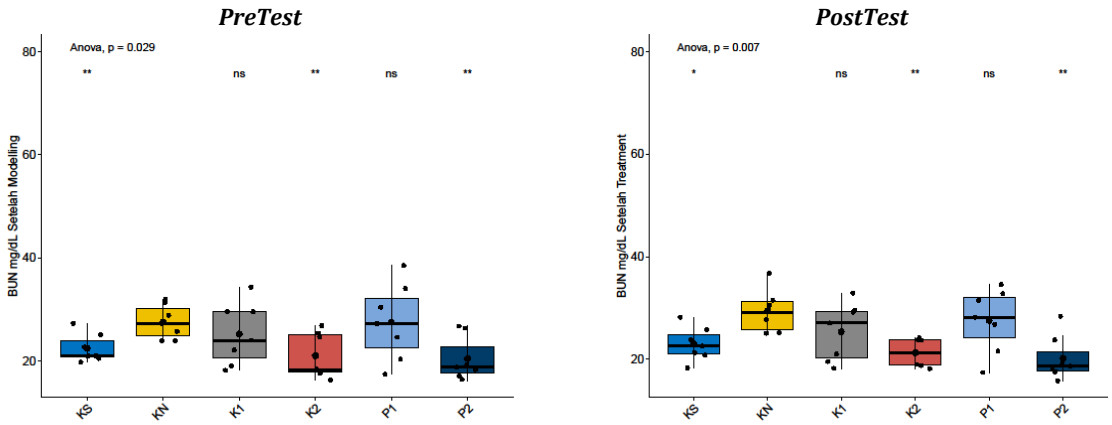


Figure 2. BUN Levels at Pre-Test and After 21-Day Intervention

The pre-test BUN levels revealed lower values in groups K1, K2, and P2 compared to KN. Following the 21-day intervention, post-test analysis demonstrated reduced BUN levels in the KS (0.53 ± 0.04 mg/dL), K1 (25.36 ± 5.72 mg/dL), K2 (21.26 ± 2.88 mg/dL), P1 (27.56 ± 6.22 mg/dL), and P2 (20.19 ± 4.36 mg/dL) groups compared to KN (29.46 ± 4.50 mg/dL). Significant differences were observed between the KN and K2 groups (p-value = 0.004) and between the KN and P2 groups (p-value = 0.010). However, no significant differences were noted between the P1 and KN groups (p-value = 0.830).

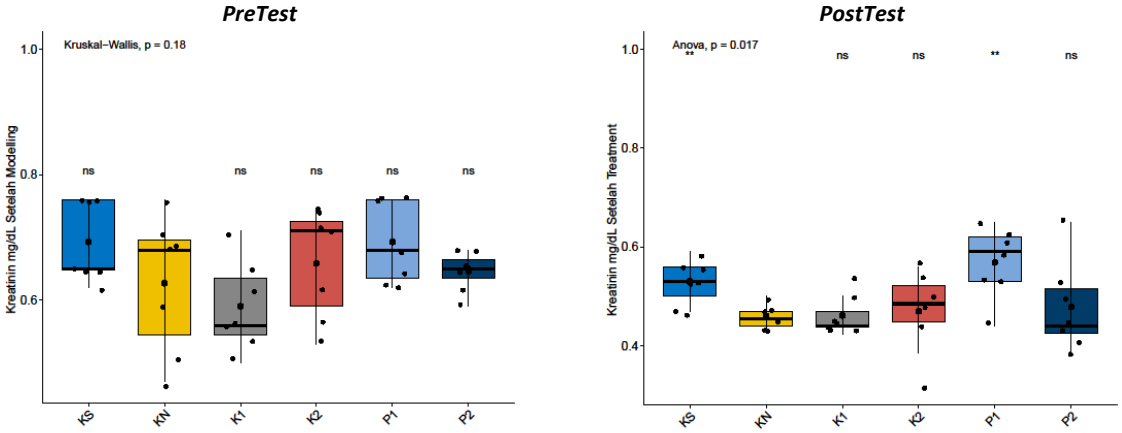


Figure 3. Creatinine Levels at Pre-Test and After 21-Day Intervention

Creatinine levels at pre-test were consistent across all groups and fell within the normal range, with no significant intergroup differences. Post-intervention analysis showed that the P1 group exhibited higher creatinine levels (0.57 ± 0.07 mg/dL) compared to KN (0.46 ± 0.02 mg/dL). Statistical tests confirmed a significant difference between KN and P1 (p-value = 0.016) and between K1 (0.46 ± 0.04 mg/dL) and P1 (p-value = 0.011). No significant difference was observed between K2 (0.47 ± 0.08 mg/dL) and P1 (p-value = 0.052).

Histopathological Analysis of Pancreatic Tissue

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

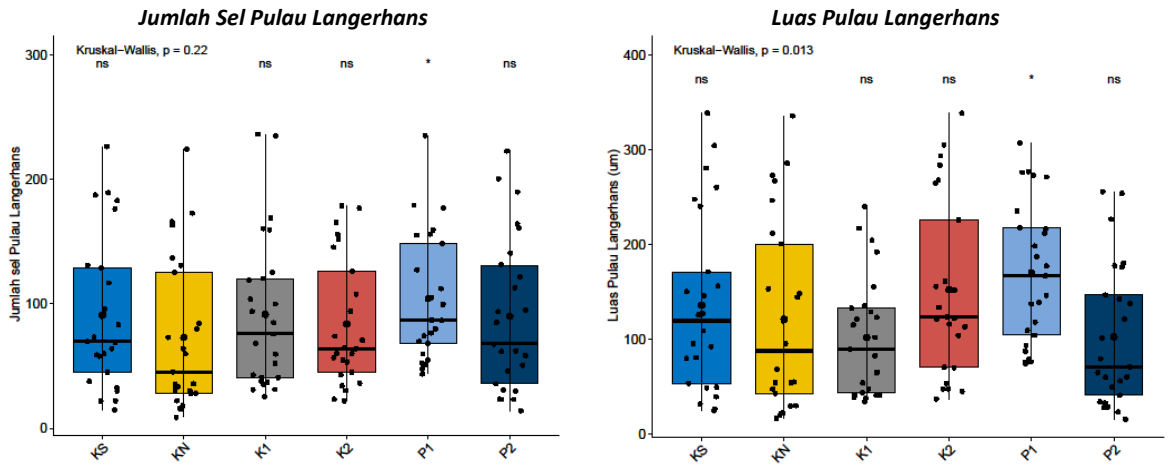


Figure 4. Islet Cell Count and Area of the Islets of Langerhans

Quantitative analysis (Figure 4) showed that the average number of islet cells in the P1 group (104.2) was higher than that in the KN group (73.04). However, Kruskal-Wallis testing revealed no statistically significant differences in islet cell counts among the groups (p-value = 0.222). In contrast, the area of the islets of Langerhans was significantly larger in the P1 group compared to KN, with a p-value of 0.013 (p-value<0.05).

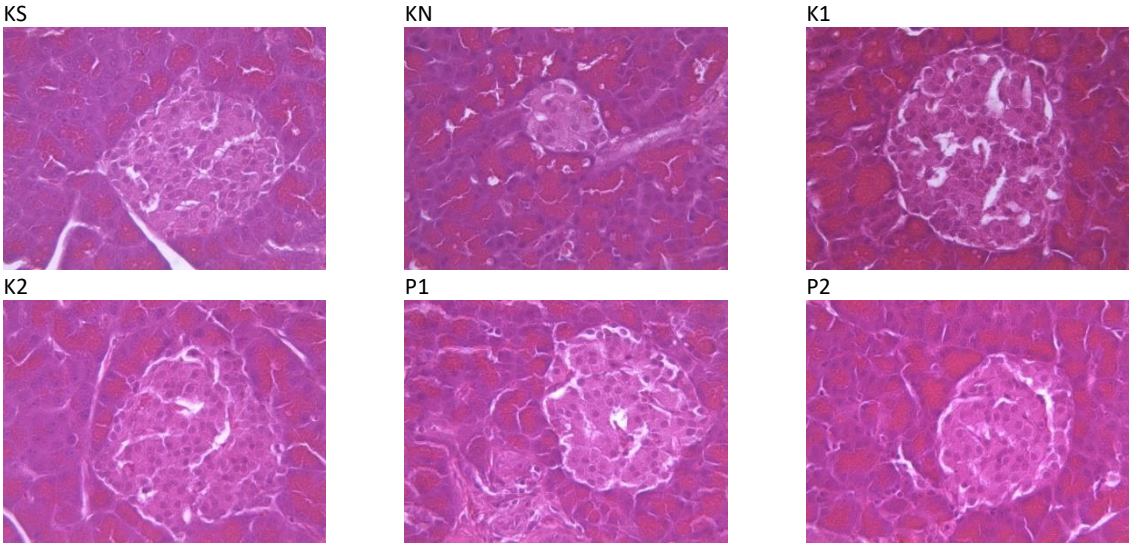
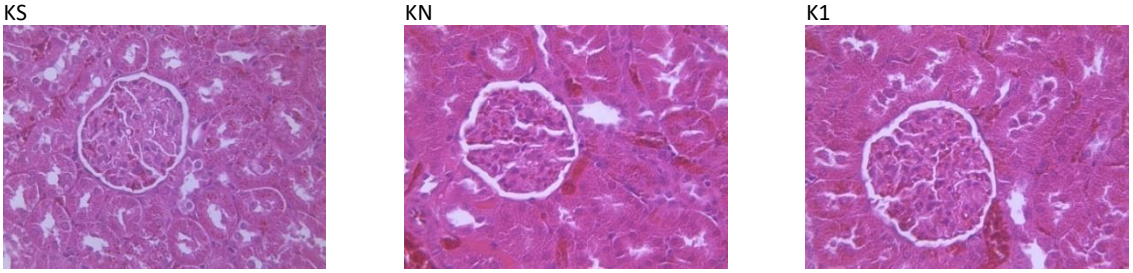


Figure 5. Pancreatic Cells Histopathology

Histological examination of the KS group revealed normal pancreatic tissue, devoid of inflammation or degeneration. The KN group exhibited pronounced damage to β-cells, characterized by reduced cell numbers and clear evidence of apoptosis. In the P1 group, administration of kefir improved the condition of the islets of Langerhans, as evidenced by increased cell numbers and expanded islet area.

Histopathology of the Kidneys



How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

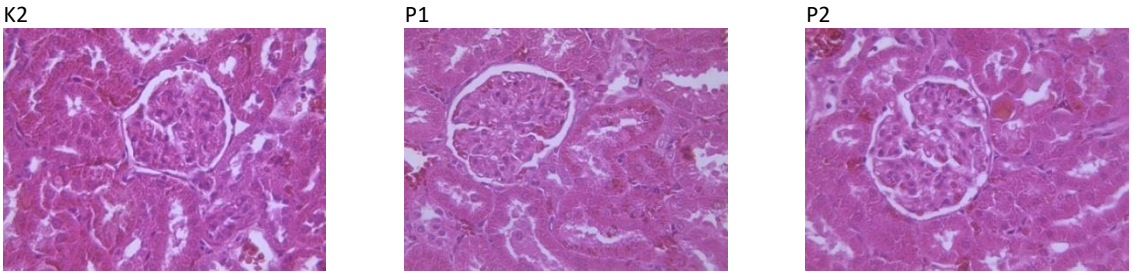


Figure 6. Histopathology of The Kidneys

Histopathological examination of kidney tissues across all experimental groups revealed no discernible abnormalities. Both the glomeruli and renal tubules appeared structurally normal, with no evidence of inflammation or necrosis. This absence of pathological changes may be attributed to the specific dosage and duration of conditioning, which were insufficient to induce significant alterations in renal tissue morphology within the Negative Control group. Consequently, the histopathological results across all groups appeared comparable. Notably, renal damage characterized by mild symptoms typically begins to manifest after 4–6 weeks in experimental diabetes models[34]. Prior studies have demonstrated that diabetic rat models exhibit histopathological features consistent with diabetic nephropathy, including glomerular enlargement indicative of hyperfiltration. These alterations are often accompanied by glomerular space dilatation and tubular epithelial damage, which collectively impair the kidneys' ability to filter waste products effectively[14].

Kefir has been shown to positively influence renal function. For instance, a study by Kahraman et al. found that kefir administration for 35 days significantly reduced BUN and blood creatinine levels in diabetic rats, alongside a notable decrease in fasting blood glucose (FBG) levels. This reduction in hyperglycemia is likely to mitigate the production of urea and creatinine[14]. Histological analyses also revealed structural improvements in renal tissues, such as reduced cast formation in renal tubules and enhanced epithelial integrity. However, the findings of this study contrast with those of Kahraman et al., suggesting that differences in conditioning and intervention durations can significantly influence outcomes. Punaro et al. similarly investigated the effects of kefir on renal function, reporting a significant reduction in BUN levels in diabetic rats treated with kefir compared to untreated controls. However, no significant differences in plasma creatinine levels were observed between the kefir-treated and untreated groups[35]. Variability in outcomes across studies may also reflect differences in kefir quality, as Kahraman et al. used commercial kefir products, whereas Punaro et al. employed traditionally prepared kefir[14].

In addition to kefir, numerous studies have explored the role of honey in improving renal function. Koodathil et al. demonstrated that high doses of bitter honey (200 mg/kg BW and 400 mg/kg BW) reduced blood urea and creatinine levels in diabetic rats induced with streptozotocin-nicotinamide[36]. Similarly, a 56-day study showed significant reductions in creatinine levels in diabetic rats treated with honey compared to untreated controls[37]. The group of diabetic rats given only honey (Tualang honey) showed some improvement in urea and creatinine levels. However, the results were more pronounced when combined with antidiabetic drugs such as metformin or glibenclamide[38].

Differences in pancreatic histopathology were observed between the Negative Control group and other experimental groups. Pancreatic β-cell damage induced by streptozotocin occurs via GLUT-2 glucose transporters on β-cell membranes, triggering oxidative stress and free radical production. These free radicals, such as superoxide anions (O2-), cause cellular damage, apoptosis, and acute inflammation in pancreatic tissues[39]. Consistent with these findings, Nurliyani et al. reported that healthy pancreatic tissue features dense islets of Langerhans with abundant β-cells, whereas diabetic states are marked by significant structural damage, including reduced islet density and diminished β-cell populations. However, in the kefir-treated group, there was an increase in the average number of Langerhans cells and β cells in diabetic rats fed goat's milk kefir for 35 days[15]. Kefir supplementation has demonstrated regenerative effects on pancreatic β-cells. A study by Handayani et al. highlighted that varying doses of goat milk kefir (250 mg/kg BW, 500 mg/kg BW, and 750 mg/kg BW) promoted pancreatic β-cell regeneration, with the highest dose yielding the most pronounced effects[40]. Similar benefits were observed with high doses of bitter and Manuka honey, which enhanced islet size and β-cell populations in diabetic experimental rats[36,41].

Kefir is a probiotic-rich fermented beverage containing lactic acid bacteria, acetic acid bacteria, and yeast[42]. Probiotics play an essential role in the fermentation process of kefir, contributing to the final product, including overall taste and health benefits[43]. Moreover, probiotics in kefir contribute to its health benefits, including improved kidney function. Lactobacillus and short-chain fatty acid (SCFA)-producing bacteria have been shown to enhance the secretion of glucagon-like peptide-1 (GLP-1) by upregulating receptors such as GPR43 and GPR41 in the intestines, which are activated by SCFAs. This mechanism not only promotes the production of GLP-1 but also elevates plasma insulin levels, both of which are essential for maintaining glycemic control. Additionally, probiotics contribute to the modulation of metabolic parameters associated with kidney health by reducing triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). By improving these metabolic markers, probiotics help alleviate diabetes-induced metabolic stress on the kidneys, thereby supporting renal

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

function and overall organ health[44]. Kefir exhibits notable anti-inflammatory properties by reducing inflammation, as evidenced by its ability to lower pro-inflammatory cytokines IL-1 and IL-6 in hyperglycemic Wistar rats. Concurrently, kefir enhances the production of anti-inflammatory cytokines, such as IL-10, which helps to balance the immune response and further mitigate inflammation[45]. Honey contains a diverse array of antioxidant compounds, including phenols, flavonols, and flavones, which provide protective effects against free radical-induced damage in the kidneys[46,47]. Honey also plays a significant role in the protection and regeneration of pancreatic β-cells through various mechanisms. Its potent antioxidant and anti-inflammatory properties help mitigate oxidative stress, a primary contributor to pancreatic β-cell damage. Furthermore, honey demonstrates the potential to enhance pancreatic β-cell regeneration by upregulating the expression of key transcription factors such as MAFA, PDX-1, INS-1 and INS-2, which are critical for β-cell proliferation, differentiation, insulin production, and secretion[41,48].

This study offers a notable advantage by employing multiple parameters, including BUN, creatinine, and histopathology levels, to comprehensively evaluate the effects of kefir combined with honey on kidney function. However, certain limitations should be acknowledged. The loss of samples during the post-test phase may have impacted the reliability of statistical analyses. Additionally, the duration of the conditioning and intervention periods might have been insufficient to fully capture the effects on kidney tissue. Further, more detailed statistical analyses of renal histopathology data are required to provide a more precise and more definitive understanding of the intervention's impact on renal tissue morphology.

CONCLUSIONS

This study highlights the potential of goat milk kefir with added honey as a preventive agent for maintaining BUN levels within the normal range. Additionally, kefir enriched with honey demonstrated curative properties in enhancing kidney function, evidenced by a reduction in creatinine levels compared to the negative group, and exhibited superior effectiveness over quercetin in lowering creatinine, because it is able to reduce creatinine levels but still within the normal range, unlike quercetin. Furthermore, the combination improved pancreatic β-cells and expanded the pancreatic islet area, indicating its broader therapeutic benefits.

ACKNOWLEDGEMENT

The authors extend their sincere gratitude to Universitas Pembangunan Nasional "Veteran" Jakarta for providing grant funding, enabling the successful completion of this research. Appreciation is also expressed to the journal editors and reviewers for their valuable feedback and suggestions in refining this manuscript.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflicts of interest associated with this article. This research was supported by DRTPM KEMENDIKBUD and the Institute for Research and Service of Universitas Pembangunan Nasional "Veteran" Jakarta under contract number 002/UN.61.4/PD-PFR/2024.

AUTHOR CONTRIBUTIONS

ALM: conceptualization, methodology, formal analysis; IMBI: conceptualization, methodology, supervision, validation, resources & Funding Acquisition, writing-review & editing, dan supervision; AH: resources & Funding Acquisition dan supervision.

REFERENCES

1. IDF. *IDF Diabetes Atlas 2021 _ IDF Diabetes Atlas*. *IDF official website* (2021).
2. BADAN KEBIJAKAN PEMBANGUNAN KESEHATAN. *Survei Kesehatan Indonesia (SKI)*. (2023).
3. Galicia-Garcia, U. *et al.* Pathophysiology of Type 2 Diabetes Mellitus. *Int. J. Mol. Sci.* **21**, 6275 (2020)https://doi.org/10.3390/ijms21176275.
4. Wickramasinghe, A. S. D., Attanayake, A. P., Wijesiri, T. W., Peiris, H. H. & Mudduwa, L. K. B. Pancreatic and Hepatic Histopathology of High-Fat Diet Fed Streptozotocin-induced Wistar Rat model of type 2 diabetes mellitus. *Ceylon J. Sci.* **53**, 235–242 (2024)https://doi.org/10.4038/cjs.v53i2.8274.
5. WHO. Diabetes. https://www.who.int/health-topics/diabetes#tab=tab_1.
6. Chutani, A. & Pande, S. Correlation of serum creatinine and urea with glycemic index and duration of diabetes in Type 1 and Type 2 diabetes mellitus: A comparative study. *Natl. J. Physiol. Pharm. Pharmacol.* **7**, 1 (2017)https://doi.org/10.5455/njppp.2017.7.0515606052017.
7. Rachmad, B. & Setyawati, R. Gambaran Kadar Kreatinin Dan Ureum Pada Penderita Diabetes Mellitus. *J. Med. Lab.* **2**, 37–45 (2023)https://doi.org/10.57213/medlab.v2i2.194.
8. Al-Mahmood, S. M. *et al.* A comprehensive study of chronic diabetes complications in streptozotocin-induced diabetic rat. *Makara J. Heal. Res.* **20**, (2016)https://doi.org/10.7454/msk.v20i2.5889.
9. Alkhatib, A. *et al.* Functional foods and lifestyle approaches for diabetes prevention and management. *Nutrients* **9**, 1–18 (2017)https://doi.org/10.3390/nu9121310.
10. Mohamed A. Farag, Suzan A. Jomaa, A. A. E.-W. & El-Seedi, H. R. The Many Faces of Kefir Fermented Dairy Products : *Nutrients* **12**, 346 (2020).
11. Dos Santos, W. M., Guimarães Gomes, A. C., De Caldas Nobre, M. S., D. S. & Pereira, Á. M., Dos Santos Pereira, E. V., Dos Santos, K. M. O., et al. Goat milk as a natural source of bioactive compounds and strategies to enhance the amount of these beneficial components. *Int. Dairy J. (Int. Dairy J.)* **137**, (2023).

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

12. Andrada, E., Marquez, A., Russo, M., Gauffin-Cano, P. & Medina, R. Fermented goat milk as a functional food for obesity prevention or treatment: a narrative review. *Front. Food Sci. Technol.* **3**, 1–6 (2024)https://doi.org/10.3389/frfst.2023.1329037.
13. Hardiansyah, A. IDENTIFIKASI NILAI GIZI DAN POTENSI MANFAAT KEFIR SUSU KAMBING KALIGESING. *J. Nutr. Coll.* **9**, 87–93 (2020).
14. Kahraman, M., Ertekin, Y. H. & Satman, İ. The Effects of Kefir on Kidney Tissues and Functions in Diabetic Rats. *Probiotics Antimicrob. Proteins* **13**, 375–382 (2021)https://doi.org/10.1007/s12602-020-09698-9.
15. Nurliyani, Harmayani, E. & Sunarti. Antidiabetic Potential of Kefir Combination from Goat Milk and Soy Milk in Rats Induced with Streptozotocin-Nicotinamide. *Korean J. Food Sci. Anim. Resour.* **35**, 847–858 (2015)https://doi.org/10.5851/kosfa.2015.35.6.847.
16. Chen, L., Bagnicka, E., Chen, H., dan Shu, G. Health potential of fermented goat dairy products: composition comparison with fermented cow milk, probiotics selection, health benefits and mechanisms.itle. *Food Funct. (Food Funct.)* **14**, 3423–3436 (2023).
17. Hardiansyah, A. & Kusuma, H. H. Optimalisasi Kualitas Organoleptik Dan Aktivitas Antioksidan Kefir Susu Kambing Dengan Penambahan Madu Lokal Bunga Randu. *J. Nutr. Coll.* **11**, 278–284 (2022)https://doi.org/10.14710/jnc.v11i4.34506.
18. Nazir, L. *et al.* Comparison of glycaemic response to honey and glucose in type 2 diabetes. *J. Pak. Med. Assoc.* **64**, 2–5 (2014).
19. Bobiş, O., Dezmirean, D. S. & Moise, A. R. Honey and Diabetes: The Importance of Natural Simple Sugars in Diet for Preventing and Treating Different Type of Diabetes. *Oxid. Med. Cell. Longev.* **2018**, (2018)https://doi.org/10.1155/2018/4757893.
20. Kunaedi, A., Aprianty, S. & Falya, Y. PENGARUH MADU HUTAN TERHADAP KADAR GULA DARAH MENCIT PUTIH (Musmuscullus) JANTAN YANG DIINDUKSI ALOKSAN. *J. Pharmacopolium* **6**, 1–7 (2024)https://doi.org/10.36465/jop.v6i2.1208.
21. WHO. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine World Health Organization. 1–73 (2000).
22. Zaki, I., Johan, A. & W, N. S. Pengaruh pemberian jus mangga terhadap profil lipid dan malondialdehyde pada tikus yang diberi minyak jelantah. *J. Gizi Indones. (The Indones. J. Nutr.* **3**, 108–115 (2016)https://doi.org/10.14710/jgi.3.2.108-115.
23. Guo, X. xuan, Wang, Y., Wang, K., Ji, B. ping & Zhou, F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. *J. Zhejiang Univ. Sci. B* **19**, 559–569 (2018)https://doi.org/10.1631/jzus.B1700254.
24. Moroti, C., Souza Magri, L., De Rezende Costa, M., Cavallini, D. C. U. & Sivieri, K. Effect of the consumption of a new symbiotic shake on glycemia and cholesterol levels in elderly people with type 2 diabetes mellitus. *Lipids Health Dis.* **11**, 1–8 (2012)https://doi.org/10.1186/1476-511X-11-29.
25. Firdaus. Peran ekstrak nutrasetikal galohgor untuk mengatasi resistensi insulin pada tikus diabetes yang diinduksi streptozotocin (stz) firdaus. (2016).
26. Edremitlioğlu, M., Andiç, M. F. & Korkut, O. Quercetin, a powerful antioxidant bioflavonoid, prevents oxidative damage in different tissues of long-term diabetic rats. *Balkan Med. J.* **29**, 49–55 (2012)https://doi.org/10.5152/balkanmedj.2011.002.
27. Ratnaningtyas, N. I. & Ekowati, N. Perdanawati AL, Ratnaningtyas NI, Hernayanti. Potensi Ekstrak Etil Asetat Coprinus comatus terhadap Kadar Ureum dan Kreatinin pada Tikus Putih Model Diabetes. Jurnal Ilmiah Biologi Unsoed. 2021;3(3):132-141. *… J. Ilm. Biol. …* **3**, 96–104 (2021).
28. Dai, K. L. Hubungan Kadar Glukosa terhadap Perubahan Kadar Asam Urat, Ureum, dan Kreatinin Serum Penderita Diabetes Melitus Tipe 2 di Malang Raya. (Universitas Islam Malang, 2020).
29. Rafe, M. A. S. R., Gaina, C. D. & Ndaong, N. A. Gambaran Histopatologi Ginjal Tikus Putih (Rattus norvegicus) Jantan yang Diberi Infusa Pare Lokal Pulau Timor. *J. Vet. Nusant.* **3**, 61–73 (2019).
30. Nugroho, P. S. *Analisis Data Penelitian Bidang Kesehatan*. (Gosyen Publishing, Sleman, 2020).
31. Han, Z.-Z. *et al.* Reference Data of the Main Physiological Parameters in Control Sprague-Dawley Rats from Pre-clinical Toxicity Studies. *Lab. Anim. Res.* **26**, 153 (2010)https://doi.org/10.5625/lar.2010.26.2.153.
32. Cahyani, E. D. *et al.* In vivo nephroprotective effect of herbal plants towards gentamicin-induced nephrotoxicity: A literature review. *J. Ilm. Farm.* **18**, 178–191 (2022)https://doi.org/10.20885/jif.vol18.iss2.art17.
33. Hasabi, I. S., Kardkal, B. L. & Bande, U. S. the Role of Duration of Diabetes in the Development of Nephropathy. *J. Evid. Based Med. Healthc.* **4**, 6017–6021 (2017)https://doi.org/10.18410/jebmh/2017/1212.
34. Sulistyoningrum, E. Model Hewan Coba untuk Studi Praklinik Diabetes Mellitus dan Komplikasi pada Ginjal serta Luka Diabetik. **2**, 174–186 (2024)https://doi.org/10.20885/bikkm.vol2.iss2.art9.
35. Punaro, G. R. *et al.* Kefir administration reduced progression of renal injury in STZ-diabetic rats by lowering oxidative stress. *Nitric Oxide - Biol. Chem.* **37**, 53–60 (2014)https://doi.org/10.1016/j.niox.2013.12.01

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*

2.
36. Koodathil, J., Venkatachalam, G. & Bhaskaran, K. In vitro and in vivo antidiabetic activity of bitter honey in streptozotocin-nicotinamide-induced diabetic Wistar rats. *J. Med. Life* **2023**, 91–100 (2023)https://doi.org/10.25122/jml-2022-0099.
37. Obia, O., Odum, J. & Chuemere, A. Nephroprotective and Antihyperlipedemic Activity of Honey in Alloxan Induced Diabetic Wistar Rats. *Int. J. Biochem. Res. Rev.* **22**, 1–7 (2018)https://doi.org/10.9734/ijbcrr/2018/41585.
38. Erejuwa, O. O. *et al.* Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. *Int. J. Biol. Sci.* **7**, 244–252 (2011)https://doi.org/10.7150/ijbs.7.244.
39. Beandrade, M. U., Amelia, R. & ... Gambaran Histologi Pankreas Tikus dengan Diabetes Melitus Tipe 2 yang Diberikan Tablet Kedelai Detam II. *J. Kedokt. dan …* 240–248 (2022).
40. Handayani, T. W. *et al.* PENGARUH KEFIR EKSTRAK BUNGA ROSELLA TERHADAP HISTOPATOLOGI PANKREAS TIKUS PUTIH JANTAN YANG DIINDUKSI STREPTOZOTOCIN. (2022).
41. Iftikhar, A. *et al.* Pancreatic regenerative potential of manuka honey evidenced through pancreatic histology and levels of transcription factors in diabetic rat model. *Heliyon* **9**, e20017 (2023)https://doi.org/10.1016/j.heliyon.2023.e20017.
42. Rosa, D. D. *et al.* Milk kefir: Nutritional, microbiological and health benefits. *Nutr. Res. Rev.* **30**, 82–96 (2017)https://doi.org/10.1017/S0954422416000275.
43. Sulmiyati, Said, N. S., Fahrodi, D. U., Malaka, R. & Maruddin, F. The physicochemical, microbiology, and sensory characteristics of Kefir Goat Milk with different levels of Kefir Grain. *Trop. Anim. Sci. J.* **42**, 152–158 (2019)https://doi.org/10.5398/tasj.2019.42.2.152.
44. Wang, Y. *et al.* Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice. *Biomed. Pharmacother.* **125**, 109914 (2020)https://doi.org/10.1016/j.biopha.2020.109914.
45. Hadisaputro, S., Djokomoeljanto, R. R., Judiono & Soesatyo, M. H. N. E. The effects of oral plain kefir supplementation on proinflammatory cytokine properties of the hyperglycemia Wistar rats induced by streptozotocin. *Acta Med. Indones.* **44**, 100–104 (2012).
46. Imtara, H., Al-Waili, N., Bakour, M., Al-Waili, W. & Lyoussi, B. Evaluation of antioxidant, diuretic, and wound healing effect of Tulkarm honey and its effect on kidney function in rats. *Vet. World* **11**, 1491–1499 (2018)https://doi.org/10.14202/vetworld.2018.1491-1499.
47. El-Haskoury, R. *et al.* Diuretic activity of carob (Ceratonia siliqua L.) honey: Comparison with furosemide. *African J. Tradit. Complement. Altern. Med.* **12**, 128–133 (2015)https://doi.org/10.4314/ajtcam.v12i4.19.
48. Kawamori, D. *et al.* Oxidative Stress Induces Nucleo-Cytoplasmic Translocation of Pancreatic Transcription Factor PDX-1 Through Activation of c-Jun NH2-terminal Kinase. *Diabetes* **52**, 2896–2904 (2003)https://doi.org/10.2337/diabetes.52.12.2896.

How to cite: *Mulyadewi, A. L., Ilmi, I. M. B., & Hardiansyah, A. (2024) Effects of Honey Goat Milk Kefir on Pancreatic Histopathology and Renal Function in Diabetic Rats: Efek Pemberian Kefir Susu Kambing Madu pada Gambaran Histopatologi Pankreas dan Fungsi Ginjal pada Tikus Diabetes. Amerta Nutrition, 8(3SP), 412–421.*