Trop J Nat Prod Res, July 2024; 8(7):7650-7657 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Propolis Extract Alters the Abundance of *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii*, and Ileum Mucosal Structure of Male Wistar Rats with High-Fat Diet

Ronny Lesmana[1,2,3,4,5,*], Yosi Yohanes Putra Tandi[6], Imam Megantara[1,3,4], Aziiz Mardanarian Rosdianto[1,3,5,7], Hanna Goenawan[1,2,3,4,5], Andreas Christoper[8], Julia Windi Gunadi[9,10], Putri Teesa Radhiyanti[1,3,4,5], Felix Zulhendri[2,11]

[1] *Department of Biomedical Science, Faculty of Medicine, Universitas Padjadjaran, Sumedang 45363, Indonesia*
[2]*Center of Excellence in Higher Education for Pharmaceutical Care Innovation, Universitas Padjadjaran, Sumedang 45363, Indonesia*
[3]*Division of Biological Activity, Central Laboratory, Universitas Padjadjaran, Sumedang 45363, Indonesia*
[4]*Science Laboratory, Graduate School, Universitas Padjadjaran, Bandung 40132, Indonesia*
[5]*Center Study of Sport Science, Wellness, and, Longevity, Graduate School, Universitas Padjadjaran, Bandung 40132, Indonesia*
[6]*Bachelor of Medicine, Faculty of Medicine, Universitas Padjadjaran, Sumedang 45363, Indonesia*
[7]*Veterinary Medicine Program, Faculty of Medicine, Universitas Padjadjaran, Sumedang 45363, Indonesia*
[8]*PMDSU Program Batch IV, Doctoral Program, Faculty of Medicine, Universitas Padjadjaran, Bandung 40132, Indonesia*
[9]*Department of Physiology, Faculty of Medicine, Universitas Kristen Maranatha, Bandung 40164, Indonesia*
[10]*Master Program of Skin Ageing and Aesthetic Medicine, Faculty of Medicine, Universitas Kristen Maranatha, Bandung 40164, Indonesia*
[11]*Kebun Efi, Kabanjahe 22171, Indonesia*

ARTICLE INFO

Article history:
Received: 27 May 2024
Revised : 29 May 2024
Accepted : 09 July 2024
Published online 01 August 2024

ABSTRACT

High-fat diet (HFD) lifestyle contributes to global morbidity and mortality due to obesity. As a potential therapy candidate, propolis modulates gut microbiota affected by HFD through direct and indirect stimulation. This study investigates the effect of propolis on gut microbiota and ileum mucosal structure, using histological analyses to validate its efficacy in HFD intervention. Male Wistar rats were randomly assigned to one of four diet groups. Fecal microbiota analysis focusing on *B. thetaiotaomicron*, *F. prausnitzii* was conducted over several weeks using 16S rDNA sequencing. In addition, ileum mucosal changes were analyzed post-termination. The novelty of propolis intervention was observed in counteracting the burden of *B. thetaiotaomicron*, an inflammation indicator, through indirect stimulation, which led to an increase in *F. prausnitzii*. Propolis affected bacterial diversity through direct and indirect stimulation, resulting in *B. thetaiotaomicron* and *F. prausnitzii* reduced and achieved homeostasis at the end of the intervention. The overall bacterial profile showed a significantly higher relative ratio of *F. prausnitzii* to *B. thetaiotaomicron* between HFDP to NCD, NCDP ($p<0.05$). Validation through ileum histology revealed that the HFD group had reduced muscle thickness, fewer Peyer's patches, and fewer villi than the NCD group ($p<0.05$). The HFDP group showed significantly more villi and Peyer's patches than the HFD group ($p<0.05$). Propolis effectively ameliorated the dysbiotic state of the gut microbiome, helping achieve favorable homeostasis. The HFDP group showed no significant differences compared to the NCD group, indicating the protective effect of propolis on the structure and physiological functions of the gastrointestinal system.

Keywords: Dysbiosis, gut-microbiome axis, gut microbiota, high-fat diet, intestinal histology, propolis

Introduction

High-fat diet (HFD) lifestyle contributes to the global increase in morbidity and mortality due to overweight and obesity.[1] According to the World Health Organization (WHO), in 2016, an estimated 650 million adults were obese, accompanied by 340 million children between the ages of 5 and 19 who were also battling obesity.[2] Poor nutrition and lack of physical activity contribute significantly to the increase in the prevalence of overweight and obesity, reaching 5.2% in children under 5 years old, 10.5% in those aged 6-12 years old, and 7% in the 13-18 years old.[3]

*Corresponding author. Email: ronny@unpad.ac.id
Tel: +62 87736671707

Citation: Lesmana R, Tandi YYP, Megantara I, Rosdianto AM, Goenawan H, Christoper A, Gunadi JW, Radhiyanti PT, Zulhendri F. Propolis Extract Alters the Abundance of *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii*, and Ileum Mucosal Structure of Male Wistar Rats with High-Fat Diet. Trop J Nat Prod Res. 2024; 8(7):7650-7657 https://doi.org/10.26538/tjnpr/v8i7.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Obesity is caused by numerous dietary risk factors, such as excessive carbohydrates and a high-fat diet.[4] High-fat diet (HFD) alters gut microbiome diversity, leading to dysbiosis, consequently affecting endotoxin production and promoting bacterial lipopolysaccharides' translocation into the systemic circulation.[5] HFD triggers local inflammation in the digestive system by increasing the ratio of free fatty acids, which subsequently impairs physiological functions and reduces the capability of intestinal adaptation. This disrupts intestinal permeability and stability and significantly decreases goblet cells, villi quantity and size, intestinal epithelial barrier disruption, and hyperplastic mucosa lesions.[6] In recent times, many studies have demonstrated the relationships between the types of gut bacteria and metabolism.[7,8] Analyses have been conducted on the species *Bacteroides thetaiotaomicron* (phylum Bacteroidetes) and *Faecalibacterium prausnitzii* (phylum Firmicutes). Both have unique characteristics and have been demonstrated to play an essential role in overweight and obesity.[9] *B. thetaiotaomicron* increases with a high-fat diet (HFD) induction through the regulation of lipid metabolism, having detrimental effects related to overweight and obesity.[10] On the

other hand, *F. prausnitzii*, a butyrate-producing bacteria, directly correlates with anti-inflammatory effects, improved insulin sensitivity, and enhanced intestinal barrier function.[11,12]
Alternative therapies are vital for mitigating the risks associated with a high-fat diet (HFD), especially considering their multifaceted effects and minimal side effects.[13] Considering the rich biodiversity in tropical countries, particularly Indonesia, there is substantial potential for developing natural therapies such as propolis as a viable therapeutic candidate.[14] Propolis is known for its antioxidant, antimicrobial, antiviral, and anti-cancer activities.[15] The specific group of bioactive compounds in propolis, polyphenols, has not been thoroughly investigated, especially concerning inflammation induced by dysbiotic conditions in a high-fat diet (HFD).[16] In the previous study, propolis was demonstrated to modulate the levels of short-chain fatty acids (SCFAs), which might contribute to the improvement of intestinal barrier function, the repair of the intestinal mucosa, the reduction of gut permeability, the increase in villi size, and the mucosal regeneration that were negatively affected by HFD.[17] There are still limited studies regarding the effect of propolis on microbiota diversity. In this study, we examine the influence of propolis on gut microbiota through histological analysis of rats on a high-fat diet (HFD). Our aim is to assess the potential of propolis as a natural treatment for dysbiosis in those on an HFD.

Materials and Methods

Animals and treatments
A total of 40 male Wistar rats (*Rattus norvergicus*) aged eight weeks with weights ranging from 220 to 250 grams were randomly divided into 4 groups: normal chow diet (NCD), NCD with 300mg/kg BW Propolis (NCDP), HFD, HFD with 300mg/kg BW Propolis (HFDP). Each container (54 cm x 36.5 cm x 28.5 cm) held 3-4 rats. The rats are housed in a room with a stable temperature of around 22-24°C and a balanced light-dark cycle (12: 12 h), with food and water provided ad libitum. They were acclimatized for one week. All acclimatization and administration processes were conducted in the Animal Science Laboratory at the Postgraduate Facility, Universitas Padjadjaran. Figure 1 illustrates the research workflow. The ethical permission for *in vivo* study was approved by the Ethics Committee of Universitas Padjadjaran (Institution of Animal Care Use Committee (IACUC)): 792/UN6.KEP/EC/2022. The NCD and NCDP groups were fed a standard chow diet D12450J -10kcal% (Research Diets, USA), contained 67g% (70kcal%) carbohydrate, 19g% (20kcal%) protein, 4g% (10kcal%) fat. The HFD and HFDP rat groups were fed a high-fat diet D12492-60kcal% (Research Diets, USA) with 26g% (20kcal%) carbohydrate, 26g% (20kcal%) protein, 35g% (60kcal%) fat, and a maximum of 1.0% free fatty acid (palmitic acid), for 12 weeks.[18] Propolis (Karo, Indonesia) was administered to the rats via intragastric gavage at doses of 300 mg/kg body weight daily for the same 12-week period (The components of propolis have been described in a separate study).[19] After the study, the subjects were sacrified.

Selection Criteria

Inclusion Criteria
The inclusion criteria for this study were as follows: (1) male Wistar rats, (2) eight weeks of age, and (3) assessed as healthy, with no abnormalities or diseases, especially those related to the digestive and endocrine systems, such as obesity, diabetes mellitus, and cancer. Additional criteria for sample selection include: (4) fresh feces must be collected within the first two hours, preserved at a temperature of 2°C, and examined within 24-48 hours after the extraction. (5) Intestinal histological samples will be taken at the end of the procedure through an anesthesia procedure that follows adjusted guidelines, and these samples can be preserved under 2°C.

Exclusion Criteria
The exclusion criteria of this in vivo study were (1) rats with a significant increase or decrease in body weight during the adaptation process. (2) rats that experienced a weight decrease of more than 10 grams per week during treatment. (3) rats that showed a reduction in hair quantity or alterations in the color of the sclera and mouth. (4) rats that exhibit changes in the digestive tract, especially in the mouth and esophagus, during the intragastric gavage procedure.

Sample Analysis Fecal Microbiome DNA Extraction, Quantification, and Sequencing
Microbiota DNA quantification aims to detect the presence and ascertain the concentration of microbiota DNA before qPCR amplification and analysis. The 16S rDNA gene sequencing procedure was carried out at the Laboratory of the Faculty of Medicine, Maranatha Christian University (Bandung, Indonesia). The fecal microbiome was collected from 40 samples, which consisted of 10 NCD rats, 10 NCDP rats, 10 HFD rats, and 10 HFDP rats. Stool samples are preserved at 2°C. The extraction procedure was initiated by mechanical homogenization with a handheld homogenizer and vortex according to the manufacturer's instructions. DNA was extracted using the Maxwell RSC instrument 48 (Promega Corporation, USA) in conjunction with the Maxwell RSC Fecal Microbiome DNA Kit (Promega Corporation, USA). DNA concentration was measured using Quantifor Thermo Scientific Multiskan GO (Thermo Scientific Corporation, USA) dsDNA to identify the target real-time PCR concentration to be used. Quantification was carried out with standard controls. The minimum DNA concentration of each sample for analysis was 2.77ng, with a purity level > 1,70. Target template concentration in this study was using volume total DNA concentration (1µL) mixed up with nuclease-free water (NFW) (3.2µL) or volume total DNA concentration (1.25µL) mixed up with nuclease-free water (NFW) (2.95µL). Analyze occurs by using qPCR of the combination of the target template with mastermix that contains qPCR mix 5µL, Forward (F) Primer 0.4µL, and Reverse (R) Primer 0.4µL. Quantitative real-time PCR (qPCR) was carried out using the AriaMx Real-Time PCR (qPCR) instrument (Agilent, USA) and Toyobo kit (Toyobo Corporation, Japan). The 16S rDNA was amplified using primer sets. The primer sequences are shown in table (Table 1).

Table 1. Primers used for Quantitative Real-Time PCR

Species	Primer Sequence (5' to 3')	Product Size (bp)	Ref
Bacteroides Thetaiotaomicron	934F: *CATGTGGTTTAATTCGATGAT* 1060R: *AGCTGACGACAACCATGCAG*	126	[35]
Faecalibacterium prausnitzii	934F: *GGAGYATGTGGTTTAATTCGAAGCA* 1060R: *AGCTGACGACAACCATGCAC*	126	[36]

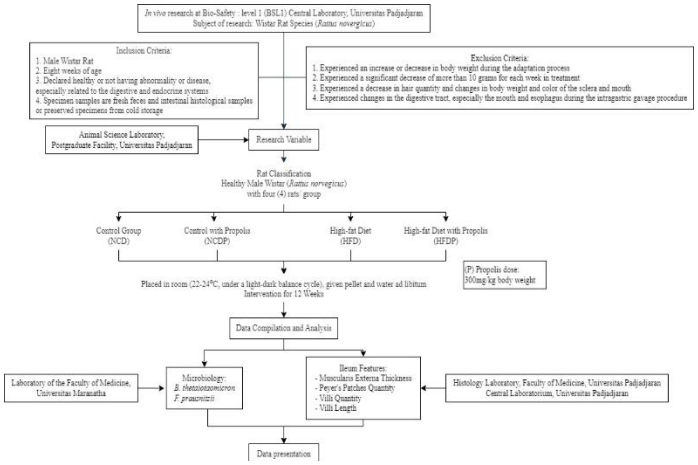

Figure 1. Research workflow

Histological analysis

The morphometric analysis of the ileum tissue was conducted using Hematoxylin and Eosin (HE) staining at the Histology Laboratory, Faculty of Medicine, Universitas Padjadjaran. After the termination, small sections of ileal tissue were fixed in 10% buffered formalin (Merck, Germany) for 24 hours. Each sample was then cross-sectionally cut to a thickness of 5µm using an MR2258 microtome (Histo-Line Laboratories, Italy) and subsequently stained with HE. Observations of the stained specimens on glass slides were performed using a ZEISS Imager Z.2 microscope (Carl Zeiss, Germany) at the Central Laboratory, Universitas Padjadjaran. The microscope was set at varying magnifications of 10x, 20x, and 40x for detailed observation and analysis. The microscopes were adjusted to 10-, 20-, and 40-times magnification for detailed observation and analysis: 10x and 20x magnification were used to define the structure of the ileum and the best structure for analysis, and 40x magnification was used for morphometric analysis, such as determining the thickness of the muscularis externae, identifying Peyer's patches, and determining the villi's characteristics. Histological analysis was performed with ImageJ 1.53t (National Institutes of Health, USA) for measurement of sample length, thickness, and volume.

Statistical analysis

All data were tabulated using Microsoft Excel 2019 software. The sampling method used in this study was random sampling with one-way ANOVA with Tukey's post hoc test using IBM SPSS Statistics 25 software (IBM, USA). A *P value* <0.05 was considered significant.

Results and Discussion

HFD in this study predominantly contained saturated fatty acids, such as lauric acid, myristic acid, palmitic acid, and stearic acid.[20] Metagenomic data showed that the HFD-fed group had higher expression levels of several metabolic genes related to immunologic response (RELMβ), gene-related bacterial adaptation system (ATP-binding cassette transporter), and genes involved in carbohydrate metabolism (phosphotransferase) compared to the control. However, the HFD-fed group exhibited higher expression levels of membrane transport and nitrogen metabolism.[21]

Propolis or bee resin contains various biochemical compounds, such as aromatic compounds, phenolic acids, and flavonoids. Flavonoids and phenolic acids are considered the main antioxidant components found in various propolis products that can help support human health by modulation of the gut microbiome.[22,23]

Food Intake Curvature

Dietary intake was analyzed daily and evaluated on the last day of each week. Our study showed that the food consumption in the NCD intervention group (NCD and NCDP) tended to be higher compared to the HFD intervention group (HFD and HFDP) in the figure 2A. To assess the effect of the intervention on the eating behavior of the rats, this significant comparison was performed. Significant differences were found between NCD and NCDP, HFD versus NCDP, and HFDP versus NCDP ($p<0.05$). However, there was no significant difference in other comparisons, e.g., HFD vs. HFDP (Figure 2B).

Propolis and HFD Impact on Microbial Diversity

The weekly analysis of microbiota gene expression shows an increase in the 4th week and a decrease in the 8th week (Figures 3A and B), respectively. In the 12th week, before termination, it is observed that the *B thetaiotaomicron* gene expression is lowest in the HFD group and highest in the NCD group, followed by the HFDP group (Figure 3A). Conversely, the *F. prausnitzii* gene expression sees a significant increase in the 4th week in the HFDP group, while no considerable changes are noted in the other groups each week. By the end of the 12th week, the HFD group had the highest relative ratio, followed by the HFDP group (Figure 3C and D). In this study, we observed an increase in *F. prausnitzii*, the known butyrate-producing bacteria, in the HFDP group. This increase is associated with anti-inflammatory activity and protection against intestinal tissue damage (Figure 3B, D). Conversely, in the HFD group, an increase in *F. prausnitzii* is hypothesized to relate to temporary compensation for insulin sensitivity by counteracting HFD-induced insulin insensitivity (Figure 3D).[12,24] In general, the increase in *B. thetaiotaomicron* expression in HFD cases could be attributed to lipid metabolism and accumulation. Accumulated adipose tissue secretes leptin and interleukin-6 (adiponectin), subsequently triggering inflammation and hepcidine production. This condition leads to dysregulatory properties in iron homeostasis.[10] The *B. thetaiotaomicron* escalation in NCDP and HFDP groups was presumed to offset bacterial suppression through increased hepcidin expression with bactericidal activity, given the iron requirement of bacteria and physiological cells through hepcidin (Figure 3A, C).[25]

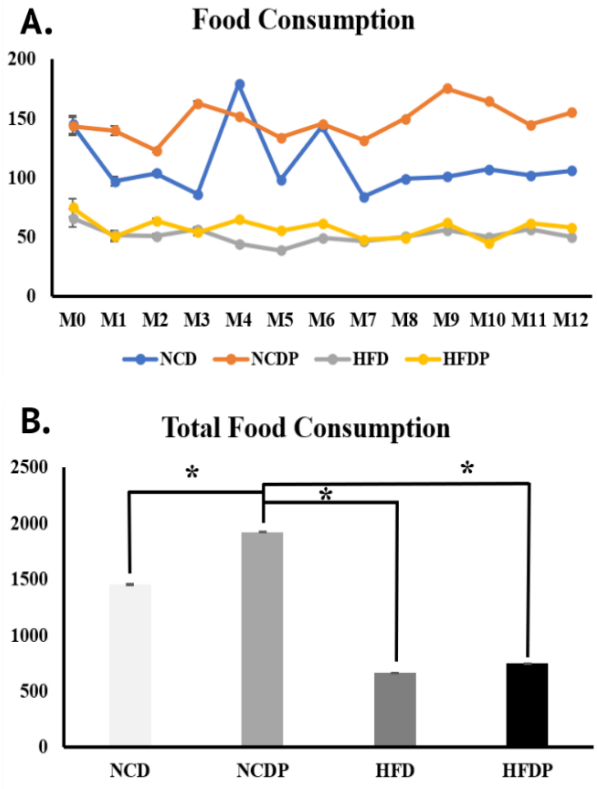

Figure 2. Eating Curvature. HFD, high-fat diet; HFDP, HFD administered propolis.

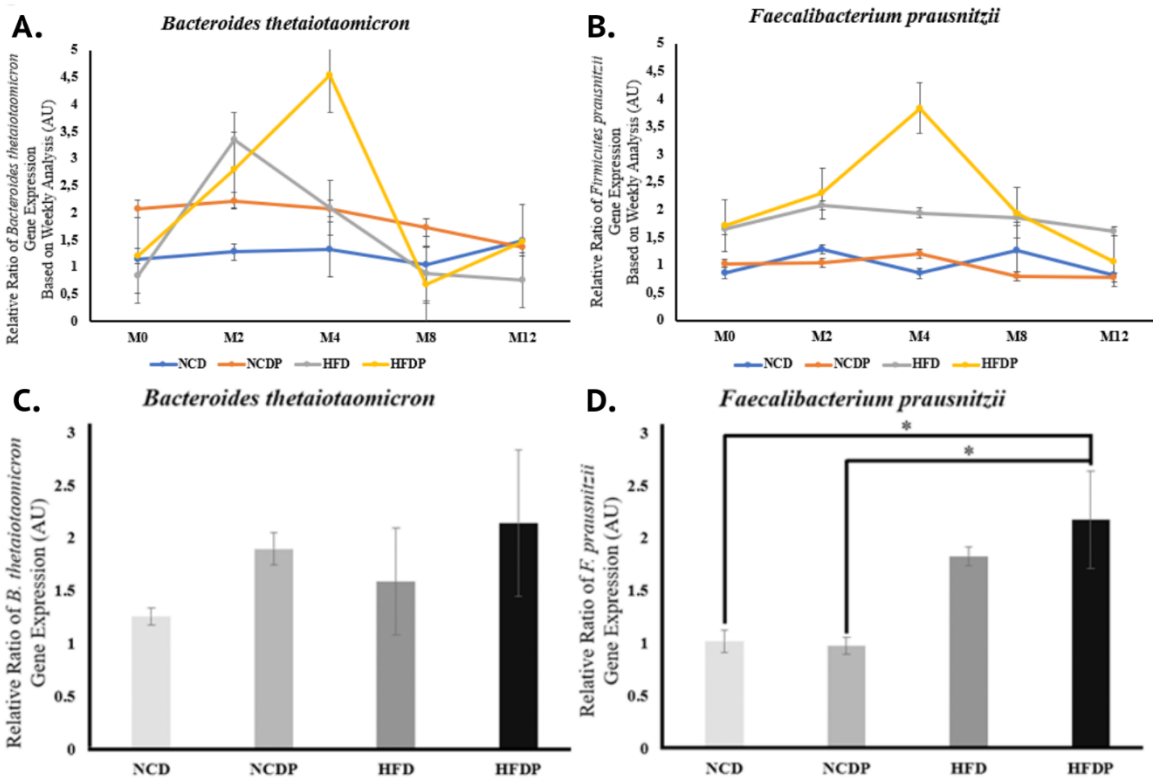

Figure 3. Analysis of the effect of HFD and propolis intervention on gut microbiota abundance. A) Analysis of changes in the expression of *B. Thetaiotaomicron* bacteria on a weekly scale. B) Analysis of changes in the expression of *F. prausnitzii* bacteria on a weekly scale. C) Relative gene expression of *B. thetaiotaomicron* bacteria. D) Relative gene expression of *F. prausnitzii* bacteria. Data presented as Mean ± SEMs. * $p < 0.05$. NCD, control; NCDP, control administered propolis; HFD, high-fat diet; HFDP, HFD administered propolis.

Nonetheless, *B. thetaiotaomicron* is directly correlated with adverse conditions such as chronic inflammation, insulin resistance, and disruption of host metabolomics.[26] Significant changes in *B. thetaiotaomicron* and *F. prausnitzii*, especially in the 4th week in the HFDP group, may represent a compensatory mechanism between bacteria, intestinal physiological conditions, and metabolism. The increased HFD burden leads to a significant increase in *B. thetaiotaomicron*. This burden is mitigated by propolis through either the inhibition of commensal bacteria or indirect stimulation, leading to a significant increase in *F. prausnitzii*. However, after the 4th week, a decline in both species was observed, visible in the 8th week, possibly caused by the direct stimulation of propolis. This modulation could potentially demonstrate the ability of propolis to re-establish the gut microbiome homeostasis instead of altering the microbiota proportion. Therefore, by the 12th week, all species increased, aligning nearly with the baseline or NCD, indicating a potential restoration of regulatory balance without causing a pathological state (Figure 3A, B). The two graphs indicate that the HFDP group has concurrently increased the expression of genes for both species. An analysis of total microbiota quantity revealed a statistically significant increase in *F. prausnitzii* gene expression in the HFDP group compared to the NCD and NCDP groups ($p<0.05$) (Figure 3D**).** This finding supports that the anti-inflammatory effect of *Faecalibacterium* may directly counter the *B. thetaiotaomicron* burden as an inflammation marker. The HFDP group exhibited the highest *F. prausnitzii* gene expression, followed by the HFD group. It has been reported that the *B. thetaiotaomicron* gene expression increased in groups that received propolis, specifically in the HFDP and NCDP groups, with the HFDP group showing the highest value (Figure 3C). Moreover, flavonoids directly and indirectly stimulate the gut microbiome. The influence of prebiotics and probiotic-like effects of propolis is thought to create a crossover effect, ameliorating the state of dysbiosis caused by HFD. This is particularly evident in the histological structure of the ileum, which is related to nutrient absorption, local and systemic inflammation, and other physiological functions.[27]

Morphometric Analysis of Ileum Tissue

The study of ileum morphological changes aimed to clarify the impact of propolis on microbiome expression and its direct effect on the intestinal system. Histopathology of the intestine, particularly the ileum, can serve as an indicator for the absorption process as well as the diversity of dominant pathogenic and/or commensal microbiota in the large intestine compared to other intestinal sections, as shown in Figure (Figure 4). The morphometry of the ileum varies among the four groups (Figure 4). No significant differences were found based on histological appearance, but quantitative analysis of each aspect showed significance. The HFD group exhibits a significant decrease in intestinal density compared to NCD in all aspects except the variation of villi length (Figure 5).

The digestive system is often referred to as a second brain. It contains a separate nervous system, the enteric nervous system, which is distinct from the central and peripheral nervous systems. In addition, the digestive system plays a role in regulating the gut microbiota. The gut microbiota can produce neurotransmitters that stimulate both the central and peripheral nervous systems. Propolis triggers the direct regeneration of the ileum through the production of biochemical compounds once the homeostasis of the intestinal microbiome has been established. This regeneration leads to the resolution of pathological damage.

The gut microbiota is also involved in immune system regulation, further impacting the nervous system. As a result, various changes in the gut microbiome directly reflect the physiological and pathological processes of the digestive system and have broader systemic implications. The observed mechanisms also have implications for various changes related to the propolis interventions, including the healing of intestinal mucosa, that crucial for intestinal mucosa regeneration, and the stimulation of intestinal-tropic glucagon-like

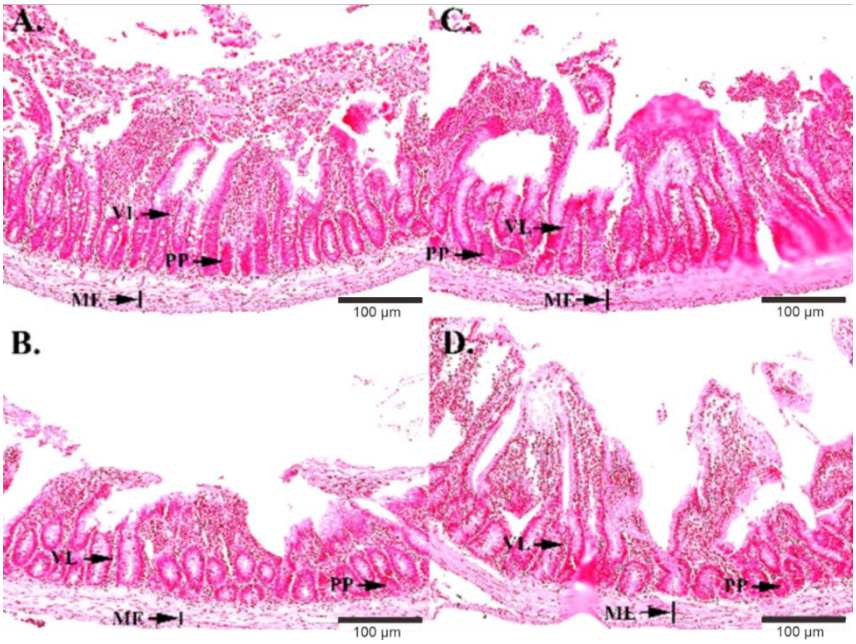

Figure 4. Representative figure of histopathological changes with microscope magnification 20X. A) NCD. B) NCDP. C) HFD. D) HFDP. ME, muscularis externa; PP, Peyer's patch; VL, villi

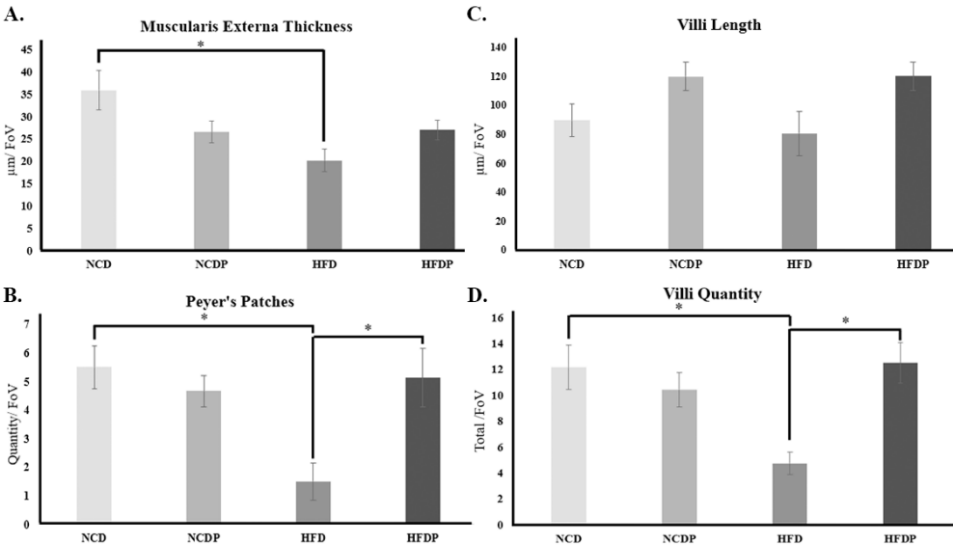

Figure 5. HFD and propolis effect on histopathological changes in several aspects. A) The effect on the thickness of muscularis externa. B) The effect on quantity of Peyer's patches. C) Changes on villi length. D) Changes in villi quantity. Data presented as mean ± SEMs. * $p < 0.05$. NCD, control; NCDP, control administered propolis; HFD, high-fat diet; HFDP, HFD administered propolis.

peptide secretion (Figure 5).[28,29] This secretion reduces gut permeability and enhances the intestinal barrier, resulting in increased expression of tight junction proteins (such as claudin, occludin, and ZO-1) and the regeneration of villi length and thickness of mucosal and intestinal epithelial tissue. The diversity of the gut microbiome directly influences the gut histological structure, potentially modulating the intestinal motility, absorption, inflammation (local and/or systemic), and gut-brain axis that reflects the systemic involvement.[30]

Muscularis Externa Thickness

The thickness of the outer muscle layer affects how the intestine moves and pushes food during digestion and is linked to direct nerve stimulation. The anatomical relationship of muscularis externa provides structural support to the villi, peyer's patch, and accessories of the intestine. Histologically, the structure of the muscularis externa in the ileum is thinner than in the duodenum, but the thickness of the villi and crypt is predominant because the physiological function of absorbing nutrients is more important than propulsion.[31] A significant difference in this thickness was noted, measuring 15.70µm per Field of View (FoV) between NCD and HFD ($p<0.05$). The HFD group had a thinner outer muscle layer compared to other groups (Figure 5A). Therefore, the reduction in the thickness of the muscularis externa aligns with the reduction of Peyer's patches and villus quantity.[32] The changes can be observed in the images (Figures 5A and D), the HFD group had significantly the lowest external muscle thickness compared to NCD, which significantly occurred in the evaluation of the quantity of Peyer's patches and villi. Indications of this dysregulation lead to reduced nutrient-absorbing function. This finding suggests better

nutrient absorption and a stronger intestinal barrier in the group with propolis intervention, limiting widespread inflammation.

Peyer's Patches Identification

Peyer's patch has a different role in intestinal cells, serving as Gut-associated Lymphoid Tissue (GALT). This function influences the transport of substances that can trigger the immune system and cause inflammation. The count of Peyer's patches in HFD rats differed from NCD rats by a mean difference of 4.00 follicles per Field of View (FoV) ($p<0.05$). A significant difference was also noted between HFD and HFDP rats, with a mean difference of 3.62 follicles/ FoV ($p<0.05$) (Figure 5B). Evidence of changes in the gut microbiota after propolis supplementation showed that a dose of 240mg/kg BW of propolis improved the structure of the mucosa and villi while decreasing inflammatory cell infiltration. In contrast, the propolis intervention of 300 mg/kg body weight in the HFDP group increased the quantity of Peyer's patches, showing a closer morphology to the control (NCD) group. This is significant as Peyer's patches were notably decreased in the HFD group compared to other groups, leading to a reduced sensitivity to antigen responses and foreign bodies, thereby making them more vulnerable to bacterial translocation and infection.[33]

Villi Properties

Villi, as projections of the intestinal wall, have a crucial role in the absorption process. Thus, the quantity and length indicate nutrient absorption and metabolism. Regarding quantity for each field of view (FoV), the NCD and HFD groups had a significant difference on average of 7.4 villi/ FoV ($p<0.05$). Significant variety was found between HFDP and HFD on an average of 7.75 villi/FoV. The HFDP groups had the highest villi quantity, followed by NCD (Figure 5C), while villi length was not significantly different for each group or relatively low compared with NCD (Figure 5D). Histological analysis of the ileum structure appears to validate the effects of a high-fat diet (HFD) and propolis interventions compared to the normal chow diet (NCD) control group. The research hypothesis posits significant differences in the histological structure between the HFD and propolis (HFDP) group and the control group. This analysis revealed significant differences in the thickness of the muscularis externa and the number of Peyer's patches between the NCD and HFD groups. Similarly, villi quantity notably varied between these groups, demonstrating that HFD intervention substantially alters the histological structure of the ileum by inducing inflammation and affecting nutrient absorption. Conversely, the HFDP group, which received both high-fat and propolis interventions, showed no significant difference from the NCD group, suggesting a return to a state closer to the normal diet. The groups that received propolis intervention (NCDP and HFDP) exhibited an increase in the quantity and length of the villi, leading to improved and optimal nutrient absorption. In contrast, the HFD group had the lowest villi quantity and length and a decreased thickness of the muscularis externa. This situation contributes to impaired food contraction and propulsion in the ileum, subsequently, food transit time will significantly increase. Importantly, inflammation was observed to have a negative correlation with the thickness of the muscularis externa.[34]

Conclusions

Direct and indirect stimulation, propolis intervention significantly contributes to achieving digestive system homeostasis. Though these interventions improved the gut microbiome, they did not fully restore it to its baseline state. This improvement, evidenced by the changes in the histological structure of the ileum, displayed no significant differences when compared with NCD, indicating restoration of a favorable structure and physiological gastrointestinal system. Despite these findings, the analysis of two bacterial species does not fully represent the gut microbiome and metabolism. Various species play distinct roles in metabolism, dysbiosis, and gut-microbiome homeostasis. In conclusion, propolis effectively ameliorates the dysbiotic state of the gut microbiome and improves intestinal function, helping achieve favorable homeostasis.

Author declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The author would like to express gratitude and high appreciation to Meita Kartika Sapitri, S.Pt (Central Laboratorium, Universitas Padjadjaran, Indonesia), who contributed significantly to the Biological Activity Division through our data compilation, research guidance, critical discussion, dan materials, and methodological assistance, and also for the provision of additional data, and research preparation. The author would like to thank several laboratory assistants such as Syafira Nurul Aini., Mia Zahrotul Munawaroh., Gilang Muhamad Nur Iqbal, M.Si., Afif Makarim Lubis, S.Si., and Mochamad Saeful Fajar, S.Si (Central Laboratory and Universitas Padjadjaran, Indonesia) and also Hesti Famella Ahsani Nissa and Agres Oktaviani (Maranatha Christian University, Indonesia) give guidance, assistance, and support related research and application of several laboratory methods. Lastly, the author would also like to thank Irfan Anis Ahmad (Histology Division of Universitas Padjadjaran, Indonesia) for preparing the histological section.
This work was supported by Indonesian Minister of Education Grant (Penelitian Fundamental-Reguler) no 3887/UN6.3.1/PT.00/2024 to HG, Internal Grant Universitas Padjadjaran (No. 1549/UN6.3.1/PT.00/2023).

References

1. Quddus A, Yimer N, Basit MA, Khan S, Amir M. Review of antioxidant-rich natural dietary products as protective and therapeutic factors against cadmium toxicity in living organisms. Pertanika J. Tropic Agric Sci.2021; 44(1):83–105. Doi:10.47836/pjtas.44.1.05
2. Vardhan KH, Kumar PS, Panda RC. A review on heavy metal pollution, toxicityand remedial measures: Current trends and future perspectives. J. Mol Liquids. 2019; 290: 111197. Doi: 10.1016/J.Molliq.2019.111197
3. Omotoso OD, Olorunnado SE, Onoja-Alexander MO, Ayodeji OS. Evaluation of the therapeutic effects of Moringa oleifera seed oil on cadmium and herbal alcoholic beverage–induced prefrontal cortex damage in Wistar rats. Asian J. Med Health. 2022; 20(12):179-187. Doi: 10.9734/AJMAH/2022/v20i12
4. Araujo-Padilla X, Briseño-Bugarín J, López-Luna A, Flores de la Torre JA. Effects ofcadmium exposure on lactatingmice and rats: A systematic reviewof breastfeeding experiments. Appl Sci. 2022; 12: 11412. Doi: 10.3390/app122211412
5. Li C, Wang B, Lu X, Huang Y, Wang H, Xu D, Zhang J. Maternal exposure to cadmium from puberty through lactation induces abnormal reproductive development in female offspring. Ecotoxicol Environ Safety. 2022; 242: 113927.
6. Unsal V, Dalkıran T, Çiçek M, Kölükçü E. The role of natural antioxidants against reactive oxygen species

produced by cadmium toxicity: A review. Adv Pharmaceu Bull. 2020; 10(2): 184-202. Doi: 10.34172/apb.2020.023
7. Nasiadek M, Skrzypińska-Gawrysiak M, Daragó A, Zwierzyńska E, Kilanowicz A. Involvement of oxidative stress in the mechanism of cadmium–induced toxicity on rat uterus. Environ ToxicolPharmacol. 2014; 38(2): Doi: 10.1016/j.etap.2014.07.007
8. Sarkar A, Ravindran G, Krishnamurthy V. A brief review on the effect of cadmium toxicity: from cellular to organ level. Intl J.Biotechnol Res.2013;3(1): 17-36.
9. Eze CE and Asomugha AL. Neurotoxicity and oxidative stress prevention by honey and garlic in adult male Wistar rats exposed to lead. Intl J.Innov Sci Res Technol. 2022; 7(1): 1011-1018.
10. Adedosu OT, Jacob AG, Alabi ZO. Protective effect of ethanol extract of Senna occidentalis leaf against cadmium-induced hepatotoxicity in rats. Trop J. Nat Prod Res. 2017; 1(1):17-21. Doi: 10.26538/tjnpr/v1i1.4
11. Tian X, Zhang K, Zhang Y, Wang N, Wang H, Xu H, Guang S. Preparation and mechanism study of hydrogen bond induced enhanced composited gelatin microsphereprobe. Intl J. Biol Macromol.2024; 266(2):130752. Doi: 10.1016/j.ijbiomac.2024.130752.
12. Ekakitie LI and Orororo OC. Changes in haematological parameters of aluminium-exposed rats treated with natural bee honey. OSR J. Environ Sci Toxicol Food Technol. 2021; 15(5): 22-25.
13. Khleifat KM, Qaralleh H, Al-limoun MO, Al-khlifeh EM, Aladaileh SA, Tawarah N, Almajali IS. Antibacterial and antioxidant activities of local honey from Jordan. Trop J. Nat Prod Res. 2021; 5(3):470-477. Doi: 10.26538/tjnpr/v5i3.10
14. Suliman RS. Efficacy of white honey in attenuating histological changes resulting from multivitamin-induced hepatotoxicity in Albino rats. Trop J. Nat Prod Res. 2021; 5(1):84-87. Doi.org/10.26538/tjnpr/v5i1.10
15. Atagana OS and Asagba SO. Protective Effects of Honey against Cadmium-Induced Alteration of some Biochemical Parameters in Rats. Toxicol Environ Chem. 2015; 96(10):1-7.
16. Diakparomre O, Onyeukwu OB, Asagba SO. Effect of honey on some biochemical parameters in the brain tissues of Wistar rats exposed to cadmium. Uniport J. Eng Sci Res. 2023; 8(1):1-8.
17. National Institute of Health (NIH). Guide for the care and use of laboratory animals. Maryland: NIH. 1985.
18. Varshney R and Kale RK. Effects of Calmodulin Antagonists on Radiation-Induced Lipid Peroxidation. Intl J. Rad Biol.1990; 58(5):733-743.
19. Reitman S and Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. Am J. Clin Pathol. 1957; 28:56-58.
20. Roy AV. Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. Clin Chem. 1970; 16:431.
21. Hillman GZ. Continuous photometric measurement of acid phosphatase activity. Zeitschrift fur KlinischeChemie und KlinischeBiochemie. 1971; 3:273-274.
22. Misra HP and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10): 3170-3175.
23. Kaplan A, Dembiec D, Cohen G, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem. 1972; 34:30-38.
24. Arbi S, Bester MJ, Pretorius L, Oberholzer HM. Adverse cardiovascular effects of exposure tocadmium and mercury alone and in combination on the cardiac tissue and aorta of Sprague–Dawley rats. J. Environ Sci Health. 2021; 56(6):609–624. Doi: 10.1080/10934529.2021.1899534
25. Singh A, Kukreti R, Saso L, Kukreti S. Oxidativestress: a key modulator in neurodegenerative diseases. Molecule. 2019; 24(8):1583. Doi: 10.3390/molecules24081583
26. Ekayoda O, Kadiri HE, Ohwokevwo OA. Combined effects of cadmium- andcyanide-contaminated diet on oxidative stressbiomarkers in different tissues of rats. Gal Med J. 2022; 29(4): E202244. Doi: 10.21802/gmj.2022.4.4
27. Kadiri HE and Apiamu A. Aframomummelegueta: a stimulator of liver function enzymes and a down-regulatorof cyanide-mediated oxidative injuries in rats. Sci World J. 2022; 17(3):375-337.
28. Al-Kafaween MA, Alwahsh M, MohdHilmi, AB, Abulebdah DH. Physicochemical characteristics and bioactive compounds of different types of honey and their biological and therapeutic properties: A comprehensive review. Antibio. 2023; 12:337. Doi: 10.3390/antibiotics12020337
29. Shalaby KA and Saleh EM. Ameliorative effect of honey bee propolis on the nonylphenol-induced reproductive toxicity in male albino rats. Aust J Basic Appl Sci. 2011;5(11): 918-927.
30. Kadiri HE. The ameliorating effects of honey on some biochemical parameters on rats exposed to cyanide. Biokem. 2018; 30(1):13-20.
31. Wang Y., Branicky R, Noë A, Hekimi S. Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. J. Cell Biol. 2018; 217(6):1915–1928. Doi: 10.1083/jcb.201708007.
32. Ezedom T, Asagba SO, Tonukari NJ. Toxicological effects of the concurrent administration of cadmium and arsenic through the food chain on the liver and kidney of rats. J. Basic Appl Zoolog. 2020; 81:16. Doi: 10.1186/s41936-020-00146-2.
33. Zayed Mohamed N, Farouk Aly H, El-Mezayen HA, El-Salamonya HE. Effect of co-administration of bee honey and some chemotherapeutic drugs on dissemination of hepatocellular carcinoma in rats. Toxicol Rep. 2019; 6(2019): 875–888.
34. Cheng N, Wu L, Zheng J, Cao W. Buckwheat honey attenuates carbon tetrachloride-induced liver and DNA damage in mice, evidence-based complement. Altern Med. 2015; 987385. Doi: 10.1155/2015/987385.
35. Nordin A, Bin Saim A, Bt Hj Idrus R. Honey ameliorate negative effects in neurodegenerative diseases: An evidence-based review. SainsMalaysiana. 2021; 50(3):791-801. Doi: 10.17576/Jsm-2021-5003-20.
36. Yaman T, Yener Z, Celik I. Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis, BMC Complement Altern Med. 2016; 16:232. Doi: 10.1186/S12906-016-1217-7.
37. Yildirim S, Celikezen FC, Oto G, Sengul E, Bulduk M, Tasdemir M, Ali Cinar D. An investigation ofprotective effects of litium borate on blood and histopathological parameters in acute cadmium-induced rats. Biol Trace Elem Res. 2018; 182:287–294.
38. Cobbina SJ, Chen Y, Zhou Z, Wu X, Zhao T, Zhang Z, Feng W, Wang W, Li Q, Wu X, Yang L. Toxicity assessment due to sub-chronic exposure to individual and mixtures of four toxic heavy metals. J. Hazard Mater. 2015; 294:109–120.
39. Yuan G, Dai S, Yin Z, Lu H, Jia R, Xu J, Song X, Li L, Shu Y, Zhao X. Toxicological assessment of combined lead and cadmium: Acute and sub-chronic toxicity study in rats. Food Chem Toxicol. 2014; 65:260–268.
40. Andjelkovic M, Djordjevic AB, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljevic J, Spasojevic-Kalimanovska V, Jovanovic M, Boricic N, Wallace D, Bulat Z. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Intl J. Environ Res Pub Health. 2019; 16(274):1-21. Doi:10.3390/ijerph16020274

41. Zhu H, Jia Y, Cao H, Meng F, Liu X. Biochemical and histopathological effects of subchronic oral exposure of rats to a mixture of five toxic elements. Food Chem Toxicol. 2014; 71:166–175.
42. Asagba SO, Adaikpoh MA, Kadiri H, Obi FO. Influence of aqueous extract of *Hibiscus sabdariffa* l. petals on cadmium toxicity in rats. Biol Trace Elem Res. 2007; 125: 47-57.
43. Abdel-Moneim WM and Ghafeer HH. The potential protective effect of natural honey against cadmium-induced hepatotoxicity and nephrotoxicity. Mansoura J.Foren Med Clin Toxicol. 2007; 15:75-98.
44. Ciriolo MR, Palamara AT, Incerpi S, Lafavia E, Bue MC, De Vito P, Garaci E, Rotilio G. Loss of GSH, Oxidative stress and decrease in intracellular pH as sequential steps in viral infection.J. Biol Chem.1997; 272(5):2700-2708.
45. Oladele JO, Adewale OO, Oyewole O, Salami MO, Owoade G, Oyeleke OM. *Annona muricata* protects against cadmium-mediated oxidative damage in the brain and liver of rats. Acta Fac. Med. Nais, . 2020; 37(3): 252-260.
46. Onwuka FC, Erhabor O, Eteng MU, Umoh IB. Ameliorative effect of cabbage extract on cadmium-induced changes on hematology and biochemical parameters of albino rats. J. Toxicol Environ Health Sci.2010; 2(2): 11-16.

