https://doi.org/10.48048/tis.2025.9331

The Future Revolution of Dadiah and Metformin to Repairing Gut Histopathology by Promoting IgA Expression of Diabetic Rats"

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Received: 15 November 2024, Revised: 23 December 2024, Accepted: 3 February 2024, Published: 15 March 2025

Abstract

Diabetic nephropathy (DN) is often associated with problems in the digestive system. Many studies have shown that probiotics contained in Dadiah play a role in improving gut health. In addition, probiotics offer therapeutic benefits when combined with metformin. There doesn't seem to be a study that looks at how Dadiah and metformin work together to improve the histopathology of the gut in a rat model of diabetes mellitus by raising the expression of immunoglobulin A (IgA). This study aimed to evaluate the synergistic effect between the combination of Dadiah and metformin in improving gut histopathology in a rat model of diabetes mellitus through increased expression of immunoglobulin A (IgA). This experimental study used adult Wistar *Rattus norvegicus* rats as the animal model. We randomly divided 35 rats into 7 treatment groups: 2 control groups and 5 treatment groups, each given a different combination of treatments. The Barthel-Manja score was used to determine what was wrong with 5 copies of each sample, and immunohistochemistry was used to determine how much IgA was in the gut tissue. Taking Dadiah and metformin together resulted in more IgA expression and significantly better intestinal histopathology scores compared to taking them separately. Combining Dadiah and metformin has the potential to usher in a new era in diabetes management. This is because both works together to improve the integrity of the intestinal epithelium by enhancing the mucosal immune response. This approach not only contributes to blood glucose control but also supports intestinal immune function, highlighting the potential of dietary intervention in comprehensive diabetes management.

Keywords: Probiotic, Dadiah, Metformin, Histopathology, IgA expression

Introduction

Currently, DN sufferers worldwide have reached 415 million people aged between 20 and 79 years. In 2040, the number of DN sufferers will continue to increase to 642 million people [1]. Meanwhile, in Indonesia in 2021, the number of DN sufferers reached around 19.47 people. This condition is already 1.5 % of the total population of Indonesia[2]. DN is the main effect of diabetes and disrupts kidney function, causing chronic, and end-stage kidney disease. Proteinuria gets worse, glomerular filtration rate (GFR) drops, and blood pressure rises all over the body. Albuminuria and swelling are the first signs [1]. Hyperglycemia has pathophysiological consequences, primarily causing microvascular changes in the kidneys[3,4]. DN generates surplus reactive oxygen species, resulting in heightened oxidative stress in organisms. When reactive oxygen species enter renal tissue, they upset the balance between oxidative and antioxidant processes. This condition can harm the glomeruli of the kidneys, make endothelial cells less effective, make blood clot more easily, change the structure of the kidneys, and lead to renal tubulointerstitial matrix fibrosis [5,6].

DN contributes to morbidity and mortality, reaching 30 % of diabetic patients [7]. DN disrupts gut health, leading to gastrointestinal (GI) problems. Diabetes can worsen sensory, motor, and secretory functions of the gastrointestinal tract in patients with type 1 and type 2 diabetes. Type 2 diabetes is commonly

associated with gastrointestinal secretory symptoms, including nausea, changes in bowel habits, and reflux [8,9]. DN can prevent GI motility and secretion [10]. Metabolic changes associated with DN can intensify GI dysfunction, affecting lipid and glucose concentrations [11]. GI can reduce the quality of life of patients with DN. On the other hand, diabetes may make gut health worse, but keeping gut health up with food and probiotics might lessen some of the negative effects and make metabolic regulation better, highlighting possible therapeutic interventions [12]. Consequently, there is an urgent need to continue improving the efficacious therapy using metformin [13,14]. Metformin significantly decreases glucose production in the liver and glucose absorption in the intestine and increases the sensitivity of peripheral tissues to insulin. In addition, metformin plays a role in increasing hypoxia-inducible factor (HIF) and autophagy in DN patients. That is why it can protect the kidney by reducing oxidative stress, endoplasmic reticulum stress, and epithelial-mesenchymal transitions [14]. Consequently, it is crucial to integrate metformin with probiotics. Combining metformin with probiotics has improved healing for patients with type 2 diabetes mellitus (T2DM) while reducing side impacts on the gastrointestinal system.

Together, metformin and probiotics have shown promise in improving treatment outcomes for people with T2DM while reducing the stomach problems that are often associated with metformin. Studies show that probiotics can help control glucose levels, improve lipid profiles, and reduce the effects of harmful bacteria in the gut. This makes them a useful addition to diabetes treatment, namely the mitigation of gastrointestinal adverse effects and enhancement of glycemic control [15]. Significant drops in glycated hemoglobin (HbA1c) levels are associated with probiotics, indicating improved long-term blood sugar control. Clinical trials involving probiotics and metformin demonstrated improvements in at least 1 glycemic measure in 64 % of cases [16]. When probiotics were taken with metformin, glycated hemoglobin (HbA1c) levels dropped significantly compared to taking metformin alone ($p < 0.05$) [17] as the study found. Probiotics increase the number of bacteria that produce short-chain fatty acids, which regulate glucose levels [16,17]. Patients who took a combination of probiotics and metformin had higher levels of butyrate synthesis, an excellent short-chain fatty acid. This helps improve glucose regulation [18]. Metformin is associated with GI complications in nearly 20 % of users, resulting in poor adherence [19]. DN patients have shown that probiotics reduce the likelihood of bloating diarrhea, and constipation; hence, improving patient compliance with metformin treatment [6]. Probiotics may improve the health of the microbiota in the gut, make insulin work better, and reduce inflammation. All of these effects may lead to better metabolic outcomes in people with T2DM [20,21].

The combination of metformin and probiotics can reduce GI disorders and improve glucose control. There was a big drop in mean FG between diabetic patients who were given metformin alone and those who were administered the medication and probiotics together (MD = −0.64, 95 % CI = −1.06, −0.22; $p = 0.003$). The combined RCTs had a substantial level of heterogeneity (I2 = 67 %). The overall certainty level of the estimate was low [22]. Administration of BB-12 probiotics with metformin therapy significantly decreased fasting glucose concentrations and HbA1c concentrations. This condition showed better control of mean differences (MD = −0.64) for fasting glucose and fewer problems with the gastrointestinal tract [23]. People with T2DM who took metformin along with probiotics had a bigger drop in HbA1c than people with T2DM who took metformin by itself (0.9 vs. 0.4 %), and they didn't have any GI symptoms. GI problems brought on by metformin were prevalent in the study population. Taking metformin in small amounts, taking prebiotics and probiotics together with it, and using it for a longer time have all been shown to protect against its GI side effects [23]. The combination of probiotics and metformin reduces GI side effects compared to metformin alone [24]. Giving metformin and Visbiome® together greatly lowers the damage that drinking alcohol does to the liver. It can also lower inflammation and oxidative stress and raise the amount of lipids in human and lab animal cell lines (human HEPG2) [25]. When given to DN patients for 12 weeks, multi-strain probiotics can boost the production of short-chain fatty acids, which can help with glucose control, GI symptoms, and insulin sensitivity [19,20]. GI problems like vomiting, diarrhea, nausea, and constipation are often linked to diabetes drugs, especially metformin, acarbose, and GLP-1 agonists [20,21]. There doesn't seem to be a study that looks at how Dadiah and metformin work together to improve the histopathology of the gut in a rat model of diabetes mellitus by raising the expression of IgA.

Dadiah is made from traditionally fermented buffalo milk. Dadiah is a fermented product from West

Sumatra, Indonesia, providing many health benefits, mostly due to its rich microbial composition, including *Lactobacillus fermentum* [26]. According to research, Dadiah functions significantly to reduce hypercholesterolemia and hyperglycemia, making it a possible treatment option for DN [**27**]. Lactic acid bacteria, namely *Pediococcus acidilactici*, play a role in producing probiotics by reducing inflammation and improving gut health. The intestine, as a very active microbiological ecosystem, is crucial in regulating the intestinal mucosal immune system [5]. IgA primarily provides adaptive humoral immune protection on the intestine's surface. IgA production in the digestive tract prevents pathogenic bacteria from attaching to intestinal epithelial cells. This prevents pathogens from entering the intestine. Researchers conducted a study to determine the concentration of IgA in the small intestine of mice treated with probiotics [8]. So, researchers have suggested that changing the gut microbiota, especially by giving probiotics, could be a beneficial alternative way to improve gut health and stop gastrointestinal disorders [9]. This study aimed to evaluate the synergistic effect between the combination of Dadiah and metformin in improving gut histopathology in a rat model of diabetes mellitus through increased expression of IgA.

Material and method

Material

This study used male Wistar rats of the *Rattus norvegicus* strain. *Rattus norvegicus* was 2 - 3 months old and weighed about 0.3 kg. *Rattus norvegicus* was healthy and active during the experiment. We used Dadiah doses of 1 and 2 mL, metformin with a single dose of 13.5 g, and Dadiah and metformin combined.

Method

We used an experimental design featuring a post-test control group. Furthermore, we administered 45 mg/kg of streptozotocin intraperitoneally to the rat model, causing it to develop diabetes. We divided 35 rats into 7 groups, namely 2 control groups and 5 treatment groups and each group consisted of 5 rats (**Table 1**). Our research has obtained ethical approval from Universitas Baiturrahmah, Padang, West Sumatra, Indonesia, with No. 136/ETIK-FKUNBRAH/ 03/09/2023.

Table 1 Division of model rat groups.

Control group		**Treatment group**				
Negative control (C−)	Positive control (C+)	Treatment group 1 (P1)	Treatment group 2 (P2)	Treatment group 3 (P3)	Treatment group 4 (P4)	Treatment group 5 (P5)
Healthy rat	Diabetic rat	Diabetic rat + Dadiah 3 g/day	Diabetic rat + Lactic Acid Bacteria (LAB) 1 mL/day	Diabetic rat + LAB 2 mL/day	Diabetic rat + Metformin 13.5 mg/day	Diabetic rat + Dadiah + Metformin

The intervention was carried out for 8 weeks; the treatment was stopped, and the intestines of the rats, such as jejunal, ileal, and colon, were cut into 1 - 2 cm pieces. We placed the rat intestine pieces in a 10 % formalin solution to further assess the inflammation score of the mouse intestinal tissue. We used hematoxylin and eosin to color 3 cross-sections of each rat intestinal sample, following the standard procedure for paraffin embedding. To assess the level of inflammation, we used the Barthel-Manja score [23] on 5 replicates of each sample. We analyzed the expression of immunoglobulin A (IgA) in intestinal tissue using immunohistochemistry (IHC) techniques [28]. This confirmed that IgA expression was present in intestinal tissue. We performed IHC using a goat-generated rat-specific anti-IgA alpha chain monoclonal antibody (Sigma Aldrich, MERCK) to visualize IgA expression in intestinal tissue. To assess the severity of inflammation, we used the Barthel-Manja combined score method. This method is based on the evaluation of 4 different histopathological parameters (**Table 2**) and produces a final score that reflects the degree of tissue damage.

Table 2 Histopathological parameters.

Histopathological parameters	A final score
Sub-mucosal edema	0; normal no pathological abnormalities
	1; mild edema (submucosal edema < 50 % of intestinal wall diameter (muscular-epithelium)
	2; moderate edema (submucosal edema 50 - 80 % of intestinal wall diameter)
	3; severe edema (submucosal edema > 80 % of intestinal wall diameter)
Leukocyte infiltration	0; < 5 PMN/high power field
	1; 5 - 20 PMN/high power field
	2; 21 - 60 PMN/high power field
	3; 61 - 100 PMN/high power field
	4; > 100 PMN/high power field
Goblet cells	0; > 28/high power field
	1; 11 - 28/high power field
	2; 1 - 10/high power field
	3; < 1/high power field
Epithelial integrity	0; normal no pathological abnormalities
	1; Epithelial Desquamation
	2; erosion (gap up to 10 epitheliums)
	3; ulceration (gap over 10 epitheliums)
Conclusion	0; Intestinal Intact/Normal without Inflammatory Signs
	1 - 2; minimal inflammation; considered normal, without signs of disease
	3 - 4; mild inflammation; considered normal, without signs of disease
	5 - 8; moderate inflammation
	9 - 13; heavy inflammation

Results and discussion"

We are done analyzing the data, which includes looking at the tissue's histopathology and measuring the amount of IgA expression using IHC methods. We present the complete results and discussion below.

Histopathology assay"

Table 3 presents the results of assessing the Inflammatory Reactive Score (IRS). The results of histopathological examination of the jejunum of each group (**Figure 1**), namely

- C− (a, h, o)
- C+ of diabetic animals (b, i, p)
- Dadiah treatment (c, j, q)
- low-dose LAB treatment (d, k, r)
- high-dose LAB treatment (e, l, s)
- metformin administration (f, m, t)
- combination treatment of metformin and Dadiah (g, n, u)

Table 3 The result of measuring the IRS.

A (Percentage of positive cells)	B (Intensity of staining)	IRS score (multiplication of A and B)
0; no positive cells	0; no color reaction	0 - 1 = negative;
1; < 10 % of positive cells	1; mild reaction	2 - 3 = mild
2; 10 - 50 % of positive cells	2; moderate reaction	4 - 8 = moderate

A (Percentage of positive cells)	B (Intensity of staining)	IRS score (multiplication of A and B)
3; 51 - 80 % of positive cells	3; intense reaction	9 - 12 = strongly positive
4; > 80 % of positive cells	Final IRS score; (A×B); 0 - 12	

Sources: [29]

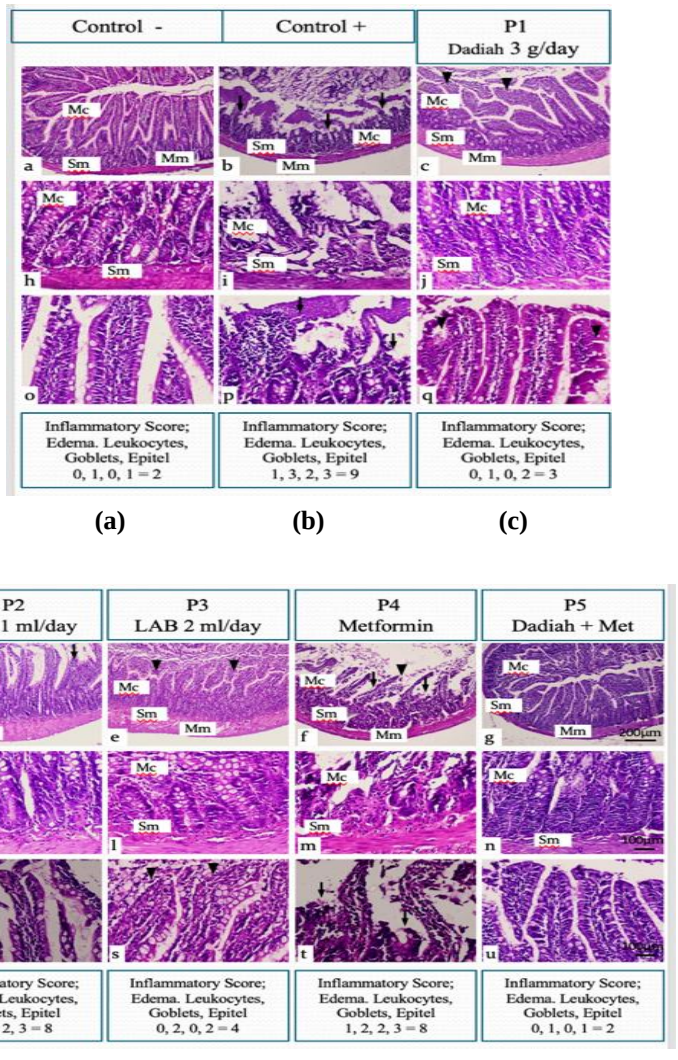


Figure 1 The result of histopathology of jejunal tissue in each group; (a) C−, (b) C+, (c) P1, (d) P2, (e) P3, (f) P4, (g) P5. Mc; Mucosa, Sm; Submucosa, Mm; Mucularis mucosa.

Mc

In **Figure 1**, the negative control group (C−) had a normal jejunal structure. The submucosa and lamina propria were not swollen, there were few white blood cells in the area but many goblet cells, and the mucosal epithelium was still whole. What was different was group C+, which had severe mucosal damage, including mucosal thinning, many ulcerations, submucosal edema, a lot of white blood cells coming in, and fewer goblet cells. Treatment groups P1 to P4 showed varying levels of mucosal damage. This damage showed up as ulcers, erosion, inflammation, and changes in the thickness of the mucosa. Group P5, on the other hand, showed improvement in the mucosa, with conditions almost similar to the negative control group: Little leukocyte infiltration, many goblet cells, and intact mucosal epithelium.

Figure 2 shows the histology of the ileum in various treatment groups. Group C had a normal ileum structure, with a lamina propria and submucosa that were not swollen, little infiltration of white blood cells,

and intact mucosal epithelium that was not rough or desquamated. However, rats in group C+ had a lot of damage to their ileum. There was mucosal atrophy, ulceration, submucosal and lamina propria edema, a lot of leukocyte infiltration, and a lower goblet cell count. Ileal damage got better with Dadiah (P1) treatment, as shown by less swelling and white blood cell infiltration, and a higher goblet cell count. However, desquamation of the mucosal epithelium was still present. Treatment with low-dose BAL (P2) showed similar results to P1 but with slightly more severe damage. Treatment with high-dose BAL (P3) showed better improvements compared to P2, with more minimal mucosal epithelial damage. Metformin administration (P4) also showed improvement in ileal damage, but there was still slight swelling and leukocyte infiltration. Using Dadiah and metformin (P5) together produced results most similar to the negative control group. The mucosal epithelial layer maintained its integrity with minimal tissue damage. Hematoxylin and eosin staining were used to observe histological preparations, with scale g: 100 µm; n and u: 100 µm.

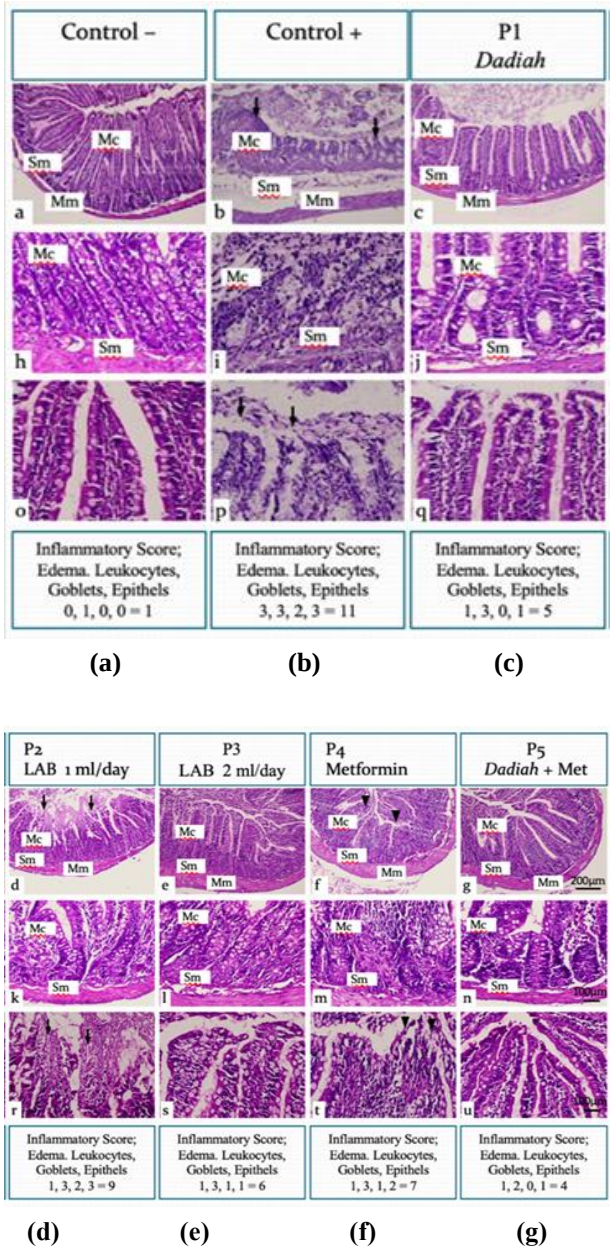
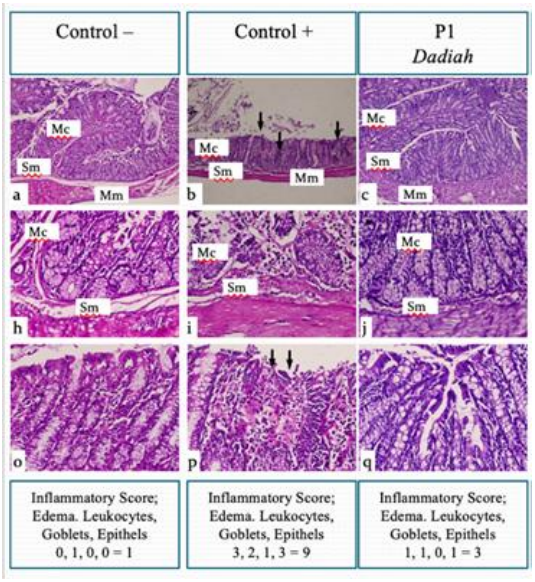


Figure 2 Histopathology of ileal tissue; (a) C−, (b) C+, (c) P1, (d) P2, (e) P3, (f) P4 and (g) P5. Mc; Mucosa, Sm; Submucosa, Mm; Mucularis mucosa.

Figure 3 shows the histological picture of the large intestine of rats in various treatment groups. The structure of the tissue in the large intestine changed with each treatment. To see this, look at groups C− (negative control) and C+ (positive control) with Dadiah (c), low-dose BAL (d), high-dose BAL (e, l, s), metformin (f, m, t), and metformin and Dadiah together (g, n, u).



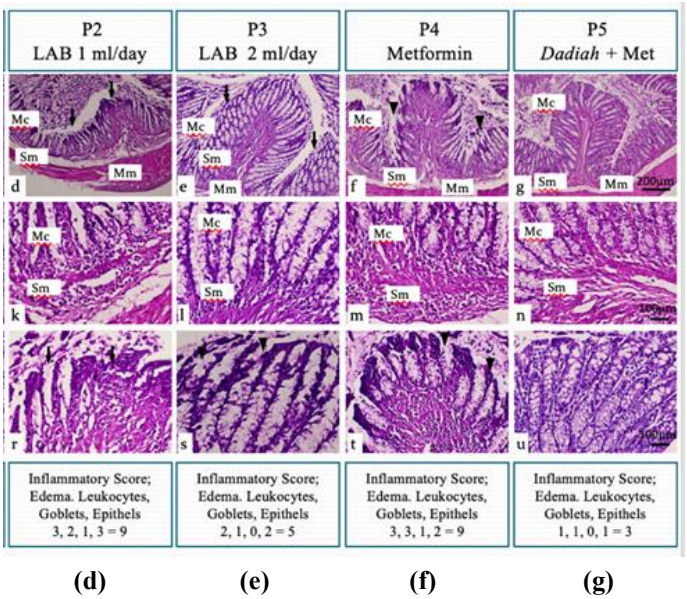


Figure 3 The histopathological of colon tissue; (a) C−, (b) C+, (c) P1, (d) P2, (e) P3, (f) P4, and (g) P5. Mc; Mucosa, Sm; Submucosa, Mm; Mucularis mucosa.

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The C-tissue looked fine; there was no swelling or increased blood flow in the lamina propria mucosa, some white blood cells were present, and there were enough goblet cells. Animals that were C+ had worse damage to their colon tissue, with sores, swelling under the mucosa and in the lamina propria, thinning of the mucosa, more white blood cells, and fewer goblet cells. In group P1, there was some swelling in the submucosal layer and a lot of white blood cells coming in, but there were still a sufficient number of goblet cells. On the other hand, there was more damage to the mucosa in group P2, with ulcers, swelling, and a lower number of goblet cells. The tissue condition in Group P3 got better, with only mild submucosal edema, low leukocyte

infiltration, and a satisfactory goblet cell count. However, in groups P4 and P5, mucosal damage was still visible with edema, decreased goblet cell count, and epithelial damage.

"Histopathology assessment based on the Barthel score"

It was clear from the Kruskal-Wallis test ($p < 0.050$) that the scores for the jejunum histopathology were very different between the groups of rats used in the study (**Table 4**). The different histopathological changes seen showed that the experimental treatment had a big effect on the diabetic rats' jejunum tissue compared to the healthy control group. These findings provide deeper insight into the mechanism of action of the treatment given.

Table 4 The result of a histopathological score of jejunal tissue varied among the groups.

"Groups	Average	Deviation standard	Notation"
C–	2.20	0.447	a
C+	8.60	0.894	d
P1	4.00	0.707	b
P2	7.40	0.548	c
P3	4.80	0.837	b
P4	7.20	1.095	c
P5	3.80	0.447	b
Kruskal Wallis		30.685	
p-value		0.000"	

Table 4 indicates that group C+ exhibited a significantly elevated mean score of 8.60 (± 0.894), although its histopathological score was considerably inferior to that of group C+. This indicates considerable jejunal dysfunction associated with diabetes. The Mann-Whitney test results demonstrated that the histopathological score of group C+ was substantially different from all other groups. Researchers may have made this discovery because diabetes greatly lowers the variety and abundance of microbiota in the jejunum. It also opens up the intestines more, which makes some infections more common [30]. We found a *p*-value of 0.000 for P1 - P5, indicating significantly less histopathological damage in all intervention groups compared to group C+. A new study backs up the idea that fermented foods could be used as medicine, especially when combined with regular treatment, to help people with diabetes deal with complications and improve their gut health, immune system response, and the integrity of the intestinal barrier. Adding probiotics to diabetic rats may improve the imbalance of bacteria in the jejunum and reduce tissue damage [31].

The combination of Dadiah and Metformin is a beneficial way to protect the damage to the digestive system. Previous studies have shown the effects of the combination of metformin with probiotics. A study of diabetic cardiomyopathy found that the combination of metformin with probiotics significantly improved heart histology [32]. Another study found how probiotics and metformin work on jejunal histopathology. Similarly, other studies have shown that metformin can mitigate radiation-induced damage to the jejunum [32]. Meanwhile, the combination of metformin and melatonin, when used alone, can prevent radiation-induced jejunal damage [33]. The combination of Dadiah and Metformin influences the breakdown and absorption of glucose. When taken together, Dadiah and Metformin can also raise the level of lactate and change the activity of glucose transporters, which can affect the body's glucose homeostasis [34,35]. The jejunum, as a place for nutrient absorption, is very responsive to this combination.

Metformin helps the jejunum absorb glucose by moving glucose transporters around, making GLUT2 work better, and blocking SGLT-1 activity [36]. The addition of Dadiah can improve the metabolic function of metformin. Metformin facilitates the passive absorption of bile salts in the jejunum, potentially

enhancing cholesterol utilization and promoting intestinal health [37]. Metformin protects against intestinal damage caused by radiation. This shows that Dadiah can reduce side effects on the jejunum caused by pharmaceutical drugs [18].

Group C had the lowest average histopathology score, which was 1.40 (± 0.894). In healthy rat, this score indicates normal histopathology of the ileum. In group C+, the average score was very high, which was 8.60 (± 0.894), meaning that diabetes caused severe damage to the ileum (**Table 5**).

"Table 5 The result of Ileal histopathology score based on Barthel score.

"Groups	**Average**	**Deviation standard**	**Notation"**
"C–	1.40	0.894	a
C+	8.60	1.817	d
P1	5.00	1.225	b
P2	7.60	2.074	cd
P3	6.40	1.140	bc
P4	5.40	1.140	b
P5	3.00	0.707	a"
"F count		17.041	
p-value		0.000"	

Research has shown that diabetic rats exhibit increased crypt depth and structural changes in the mucosal epithelial cells in the ileum. This indicates severe damage to the tissue. The ileal dysbiosis that goes along with it and the large changes in gene expression show how complicated the relationship is between diabetes and the digestive system. Knowing more about the molecular processes at play could lead to the creation of new treatments for diabetics who are having problems with their digestive systems [38].

We used analysis of variance (ANOVA) to see that the average histopathology score of the ileum was significantly different (p-value < 0.05) between the treatment groups. Duncan's post-hoc test showed that the most striking difference was in group C+, which had a significantly higher average histopathology score compared to the other groups, except for group P2. Although group C had a lower average histopathology score compared to groups P1, P2, P3 and C+, this difference was not statistically significant when compared to group P5. However, the combined use of Dadiah and metformin significantly reduced the histopathological damage. This suggests that the probiotic effects of Dadiah and the metabolic and anti-inflammatory effects of metformin may work better together. This finding is in line with existing literature [37], which suggests that probiotics can modulate the gastrointestinal side effects of metformin and enhance its efficacy.

Taking Dadiah and metformin together improved the structure of the microvilli and made the ileal mucosa thicker. More of the tight junction proteins ZO-1 and occluding were found, which meant that the intestinal barrier was stronger. In addition, metformin also plays a role in modulating the immune response by inhibiting the activation of the NF-κB pathway and thereby reducing inflammation. According to research of Chen *et al.*, this effect of probiotics and metformin working together may involve changing the makeup of the microbiota in the gut [39]. Metformin and Dadiah work synergistically to strengthen the ileal epithelial barrier [40,41] Metformin raises the levels of proteins called tight junction proteins, which help epithelial cells stick together better. It also lowers the amount of paracellular permeability. In addition, metformin also stimulates goblet cell maturation, increasing mucus production that acts as a protective layer. Because it has probiotics, Dadiah can change the microbiota in the gut, lower inflammation, and help the epithelial barrier function that metformin improves [42,43]. Barthel score analysis (**Table 6**) showed statistically significant differences in the severity of colonic histopathological damage

between groups. The control group (C) had the lowest mean score (1.20 ± 0.447), indicating normal histological conditions. The diabetic group (C+), on the other hand, had the highest mean score (8.00 ± 1.000), which meant that there was severe histological damage, such as inflammation, ulceration, or fibrosis.

Table 6 The result of colon histopathological score based on Barthel score.

” Groups	Average	Standard deviation	Notation””
C–	1.20	0.447	a
C+	8.00	1.000	bc
P1	4.20	1.304	e
P2	5.60	2.302	cd
P3	5.20	0.837	bcd
P4	6.40	2.074	de
P5	3.60	0.548	b"
“Kruskal Wallis count		24.348	
p-value”		0.000”	

A big difference ($p < 0.05$) was found in the colon histopathology scores between the treatment groups according to the Kruskal-Wallis non-parametric test. While the Mann-Whitney post hoc test showed that the C+ group had the highest average histopathology score (**Table 6**), it was also clear that this group was significantly different from the control group and all the intervention groups except group P5. These results are similar to those from earlier research [43] that showed that taking probiotics along with metformin can help fix damaged colon tissue by changing the gut microbiota and lowering inflammation. Scientists think that the probiotics in Dadiah change the gut microbiota, stop the growth of harmful bacteria, and increase the production of chemicals that reduce inflammation. By blocking the NF-B pathway, this process can enhance the anti-inflammatory effects of metformin [28]. The combination of the 2 has the potential to improve the integrity of the gut barrier and reduce the risk of diabetic complications, including damage to the colon tissue.

Mixing Dadiah probiotics with metformin improves the colon tissue of diabetic rats. Possible mechanisms involved include modulation of gut microbiota, reduction of inflammation, and enhancement of immunological responses. Studies from the past [44] back up these results by showing that the cytokine profiles of the people who were given combination therapy were significantly different. Researchers believe that this combination therapy functions by enhancing the immune environment and decreasing the colon’s inflammatory process. People who got combination therapy had significant improvements in inflammatory parameters and better control of their blood sugar [45,37]. Possible mechanisms involved include modulation of gut microbiota and increased production of anti-inflammatory metabolites. The following section outlines the main findings of the current study. Probiotics and metformin consumption significantly reduced glycated hemoglobin levels, resulting in improved blood sugar control [37]. In a pilot study, participants received both metformin and probiotics. They had lower fasting plasma glucose levels and higher production of butyrate, which is a short-chain fatty acid that is beneficial for gut health [46]. When probiotics are combined with metformin, inflammatory markers such as IL-6 and TNF-α levels are reduced. These markers are associated with diabetes and colorectal cancer [19,43]. Animal studies using histological analysis show that this combination therapy can stop tumor growth and shrink tumors, which suggests that it might be able to keep diabetics from getting colorectal cancer [45].

“The assay of IgA expression in intestinal by IHC”

We determined the concentration of IgA in rat’s jejunum tissue by immunohistochemistry. The following are the histological appearances: C− (a, h), C+ (b, i), P1 (c, j), P2 (d, k), P3 (e, l), P4 (f, m), and P5 (g, n) (**Figure 4**). We identified IgA in the stromal cells, the cytoplasm of epithelial cells, and the stromal extracellular matrix.

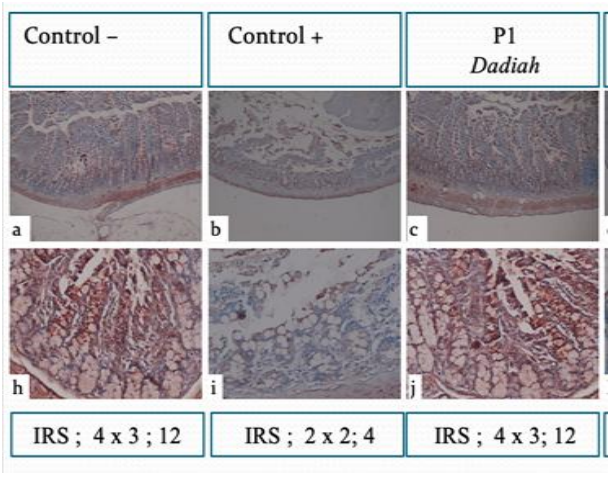

Figure 4 The IHC test looked at IgA levels in jejunal tissue; (a) C−, (b) C+, (c) P1, (d) P2, (e) P3, (f) P4, and (g) P5.

The study used histopathology to find a link between the IgA staining intensity, the number of cells that make IgA, and the thickness of the mucosa. The negative control group (C+) showed mucosal atrophy and minimal IgA expression. On the other hand, all of the parameters that were measured got better in the treatment groups (C−, P1, P4 and P5), with the percentage of IgA-positive cells being between 50 and 80 %. These findings indicate that probiotic administration can stimulate IgA production and strengthen mucosal immunity. Immunoperoxidase. Scale g: 200 µm; n: 100 µm. The following photomicrographs were used for the immunohistochemistry of rat ileum IgA tissue: C− (a, h), C+ (b, i), P1 (c, j), P2 (d, k), P3 (e, l), P4 (f, m), and P5 (g, n) (**Figure 5**).

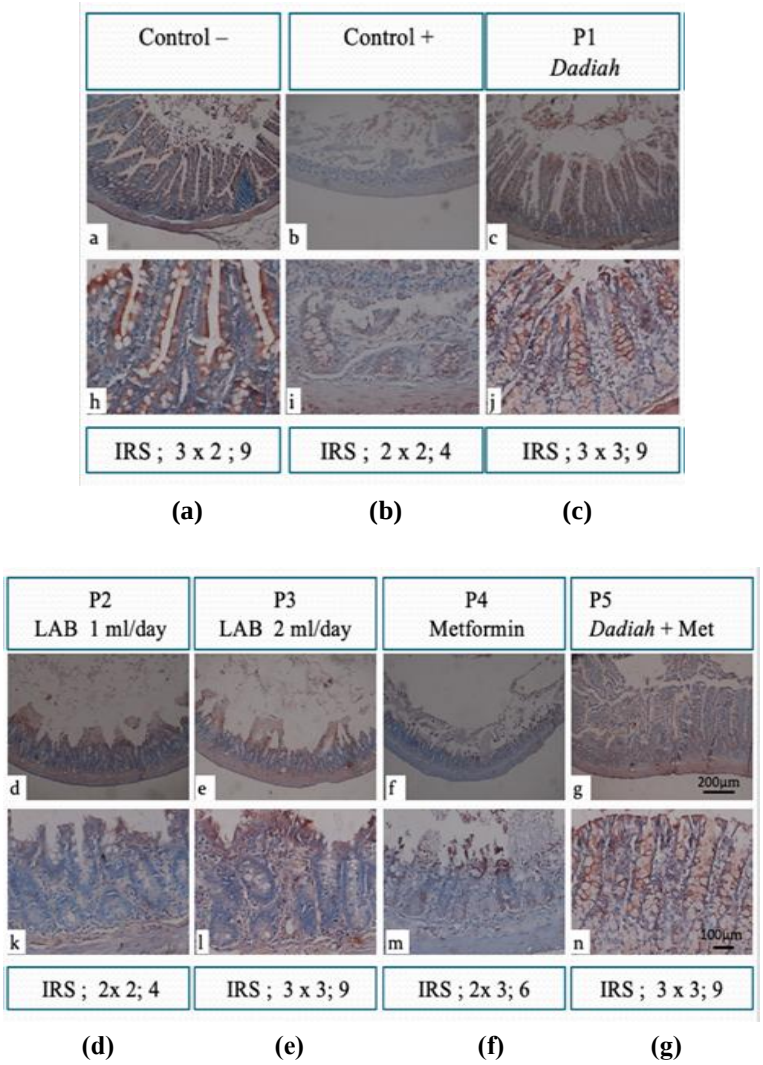

Figure 5 The IHC assessment of IgA expression in ileal tissue; (a) C−, (b) C+, (c) P1, (d) P2, (e) P3, (f) P4, and (g) P5.

Immunohistochemical analysis showed IgA expression in epithelial cells, stromal cells, and mucosal extracellular matrix. C+ rat showed mucosal atrophy and the lowest IgA expression. C−, P1, P3, and P5 mice, on the other hand, had thicker mucosa and more IgA expression (> 80 % positive cells). P2 rat showed a similar phenomenon to C+, while P4 rats showed intermediate results. A combination of Dadiah and Metformin increases IgA expression compared to C+. Immunoperoxidase. Scale g: 200 µm; n: 100 µm.

IgA immunohistochemistry was done on rat colon tissue (**Figure 6**), which shows standard areas called C− (a, h), C+ (b, i), P1 (c, j), P2 (d, k), P3 (e, l), P4 (f, m), and P5 (g, n).

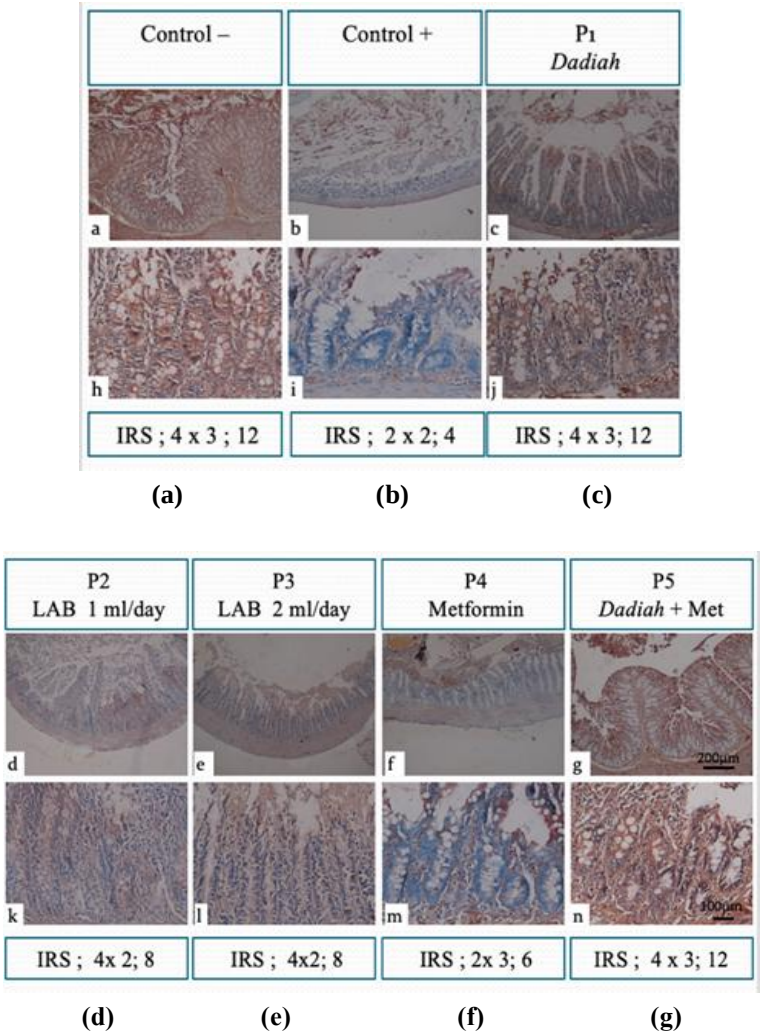

Figure 6 The IHC evaluation of IgA expression in colonic tissue; (a) C−, (b) C+, (c) P1, (d) P2, (e) P3, (f) P4, and (g) P5.

We detected IgA immunoreactivity in the cytoplasm of epithelial and stromal cells of the mucosa. C+ rat showed mucosal atrophy and the lowest IgA expression. In contrast, C−, P1, P3, and P5 rat had higher IgA expression and wider distribution. P2 rats showed a similar phenomenon to C+, while P4 rats showed intermediate results. Administration with both LABs increased IgA expression relative to C+. Immunoperoxidase. The scale is 200 µm, n; 100 µm.

The assay of IgA expression in the intestine by IHC"

IHC allows us to see the distribution of cells throughout the intestine and the cells that produce IgA (IgASC). Through IHC, cells that produce IgA (IgASC) and the distribution of cells throughout the intestine can be seen. **Table 7** shows the average IgA expression in the jejunum.

"Table 7 The result of average IgA expression in the Jejunal.

"Groups	Average	Standard deviation	Notation"
C–	9.60	2.510	a
C+	6.20	1.789	a
P1	9.20	1.643	a
P2	7.80	1.643	a

"Groups	Average	Standard deviation	Notation"
P3	7.80	1.643	a
P4"	7.20	1.643	a
P5	9.00	2.121	a
Kruskal Wallis count		9.146	
p-value		0.166	

According to this study, the jejunum had the highest level of IgA in the C therapy group, though the values were pretty spread out (**Table 7**). Although C+ therapy also showed IgA in the jejunum, the average levels were lower than some values in group C. We used the Kruskal-Wallis non-parametric statistical test to compare the treatment groups. There was no statistically significant difference in the levels of IgA in the jejunum ($p > 0.050$), which suggests that the differences in IgA levels may not be because of the different treatments. Lymphatic channels connect the ileum, the final part of the small intestine, to the colonic lymphoid tissue. Here, B plasma cells release IgA [47], an important antibody in the mucosal immune system. The ileum and colon contain scattered solitary intestinal lymphoid tissue (SILT) and gut-associated lymphoid tissue (GALT), such as Peyer's patches. These structures serve as sites for the production and proliferation of IgA-producing B cells. More than 1 study has shown that when B cells that make IgA move from the ileum to the colon, it can change the microbiota in the colon and the amount of IgA in the stool [44]. Researchers have found that chemokine receptors like CCR9 and CCR10 play a part in the movement of plasma cells that make IgA. However, it is still not clear how the specificity and diversity of IgA immune responses in different intestinal tissues are controlled. Studies have already shown that CCR9 helps IgA plasma cells get to the small intestine, and CCR10 helps them get to both the small intestine and the colon. But it's still not clear what roles these receptors play in figuring out how IgA reacts to different antigens and how the more complicated control systems work. IgA is known to be very important for maintaining intestinal health by controlling the microbiota, but no one knows where the plasma cells that make IgA come from or how they get to the colon. IgA helps maintain the balance of beneficial bacteria in the intestine and prevents the growth of pathogenic bacteria [44]. The IgA system can become dysfunctional, which can upset the balance of bacteria in the gut, raise the risk of infection, and help inflammatory bowel disease and autoimmune disorders develop [45].

We used IHC to investigate the expression of IgA in the ileum. This gave us a better idea of the immune system's health in that area and its potential for finding and keeping an eye on different diseases. **Table 8** summarizes the quantitative results of IHC analysis in each group.

Table 8 The result of IHC analysis in each group.

"Groups	Average	Standard Deviation	Notation"
"C–	8.60	0.548	c
C+	4.80	1.095	a
P1	8.40	0.548	bc
P2	8.40	0.548	bc
P3	8.40	0.548	bc
P4	7.40	1.342	b
P5"	8.40	0.548	bc
Kruskal Wallis count		16.564	
p-value		"0.011"	

The Kruskal-Wallis test showed that the 3 treatment groups had statistically different levels of ileal IgA ($p < 0.011$). Group C- showed the highest average ileal IgA levels (8.6 ± 0.548), while group C+ had the lowest average levels (4.8 ± 1.095) (**Table 8**). The treatment that was given to group C had a bigger impact on increasing IgA production in the ileum than the treatments that were administered to the other groups. Further tests, such as the Mann-Whitney test, are necessary to specifically determine which groups were significantly different. We looked at the data and found that metformin raised IgA levels in the ileum by a lot, especially when compared to the C+ control group. Combining metformin and Dadiah enhanced their ability to elevate IgA levels to normal levels in rat. Researchers believe this is due to the synergistic effect of the bioactive components in metformin and Dadiah, which enhance IgA production. More than that, giving different amounts of Dadiah isolates can also raise IgA levels, especially in the ileum. There is probably more gut-associated lymphoid tissue (GALT) in the ileum than in the jejunum, which is why there is a higher rise in IgA levels there. The study's results strongly suggest that Dadiah and metformin together could be used as an extra treatment to boost the immune system of the lining of the gut. Research shows that probiotics, especially lactic acid bacteria, can increase IgA levels through various mechanisms. To give you an example, *Lactobacillus sakei* increases the production of IgA by removing vesicle membranes that turn on TLR2 receptors on Peyer's patch cells. This makes the immune response stronger [44]. Additionally, studies have shown that *Bifidobacterium bifidum* raises IgA levels by boosting the production of APRIL [45]. It is also known that Dadiah can change the microbiota in the gut, which could make the effect of probiotics stronger in increasing IgA production. The interaction between probiotics, metformin, and gut microbiota creates an environment conducive to enhancing gut mucosal immunity [46]. To give you an example, *Leuconostoc mesenteroides* make exopolysaccharides that can help the body make more IgA and strengthen the mucosal barrier.

The colon produces IgA, which maintains intestinal health and regulates microbial activity. **Table 9** shows the result of IgA expression of the colon in each group

Table 9 The result of IgA expression of the colon in each group."

"Groups	Average	Standard Deviation	Notation"
C–	10.80	1.643	d
C+	4.80	1.095	a
P1	9.60	1.342	cd
P2	8.60	0.548	bc
P3	8.40	0.548	bc
P4	7.80	1095	b
P5	9.00	1.732	bc
Kruskal Wallis count		22.817	
p-value		"0.001"	

The C− group had the highest average colonic IgA levels (10.8 ± 1.643), which was much higher than the C+ group (4.8 ± 1.095) (**Table 9**). Kruskal-Wallis analysis demonstrated statistically significant differences in average colonic IgA levels among the groups ($p < 0.001$). Subsequent Mann-Whitney tests revealed that the C− group differed significantly from C+, P2, P3, P4, and P5, but not from P1.

We can see from these results that the Dadih intervention works to get colonic IgA levels back to what they were in healthy controls (P1). Notably, the combination of Dadih and Metformin also achieved comparable results. Our study shows that Dadih's nutritional and probiotic parts work together to improve the function of B plasma cells and the release of IgA. Researchers also think that the higher production of IgA is due to lymphoid tissue moving from the ileum to the

mucosa of the colon [47]. The C+ treatment exhibited the lowest average colonic IgA levels compared to C−, P1, P2, P3, P4, and P5. This finding aligns with the observation that elevated blood glucose levels in diabetic animals can impair mucosal IgA synthesis. Lower IgA levels weaken the barrier in the gut, which makes dysbiosis and changes in the gut microbiota easier to cause. B-lymphocytes are crucial for humoral immunity, producing serum immunoglobulins such as IgA, IgG, and IgM. Serum immunoglobulin concentrations serve as key indicators of humoral immune status.

Earlier research showed that giving piglets *Lactobacillus salivarius* by mouth increased the variety of microbiota in their guts and the number of immune cells, such as IgA-producing cells and intraepithelial lymphocytes [42,43]. These findings support the notion that probiotics can stimulate intestinal epithelial cells to enhance immunoglobulin secretion. The combination of Dadiah and metformin offers intriguing potential in improving gut health. By making more IgA, this mix may help keep you from getting GI infections, lower chronic inflammation, and absorb nutrients better. These results show that probiotic methods, like Dadiah consumption, may be a useful addition to managing diabetes. They may help lower blood sugar levels and make the digestive system healthier in general. It has been shown that certain strains of *Lactobacillus sakei* can increase the production of IgA. This is done by activating the TLR2 receptor in membrane vesicles, which improves immune responses in mouse Peyer's patch cells [47]. It has also been shown that *Leuconostoc mesenteroides* can raise IgA levels in both feces and plasma [45], which helps protect the mucosal barrier and lowers oxidative stress rates. The study found that taking both probiotics and metformin together increased the expression of intestinal tight junction proteins and decreased inflammation. This showed that IgA production and overall gastrointestinal health were better [37].

Conclusions

After receiving a combination of Dadiah and Metformin, histological observations in diabetic rats showed significant improvements. This treatment group healed a lot of intestinal mucosal lesions, like ulcers and inflammation, that are common in people with diabetes. These results demonstrate the ability of the combination therapy to repair gastrointestinal tissue damage resulting from diabetes. As more IgA is produced, the mucosa may stay intact and prevent pathogens from entering. The increase in IgA levels indicates a better immune response and a healthier intestinal environment in the treatment group. The results showed that giving diabetic rats both Dadiah and Metformin together made their intestines much healthier, as shown by a drop in inflammatory markers. In addition, this combination also has the potential to improve the health of the intestinal microbiota, which plays an important role in maintaining the body's metabolic functions, including glucose absorption. The increase in IgA levels observed in the treatment group indicates a strengthening of the mucosal immune system and protection against infection. These findings indicate that the combination therapy of Dadiah and Metformin can be a promising approach in the management of diabetes, not only to control blood sugar levels but also to improve overall gastrointestinal health. These findings show the enormous potential of the combination of Dadiah and Metformin as an adjuvant therapy in the management of diabetes. In addition to controlling blood sugar levels, this combination is also able to repair gastrointestinal tissue damage and improve mucosal immune function. We need to learn more about how this synergistic effect works, such as what role probiotics play in Dadiah and how they interact with metformin in a complex way. A deeper understanding of these mechanisms will open up opportunities for the development of more personalized treatment strategies, tailored to individual characteristics and types of diabetes. The results of this study provide new hope for the development of therapies that not only focus on glucose control but also improve the quality of life of diabetes patients through gastrointestinal health. These directions for future research show that using Dadiah and Metformin together might not only help people with diabetes but also help us learn more about gut health and how it affects metabolic diseases. Further research is needed with clinical trials in humans.

Acknowledgments

Thanks to Prof. drh. Hj Endang Purwati, MS, PhD from the Animal Biotechnology/Animal Product Technology Laboratory, Andalas University Padang, West Sumatra, Indonesia for providing research materials. Thanks to the Baiturrahmah University Foundation for helping finance this research.

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