

## HISTOPATHOLOGICAL DESCRIPTION OF OCULAR VASCULAR IN ALLOXAN-INDUCED DIABETIC RATS FED FREEZE-DRIED SOYGHURT (*GAMBARAN HISTOPATOLOGI VASKULARISASI MATA PADA TIKUS DIABETIK YANG DIINDUKSI ALLOXAN DAN DIBERIKAN SOYGHURT BEKU-KERING*)

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### ABSTRACT

Diabetes mellitus is a metabolic disease that can cause hyperglycemia (elevated blood sugar levels). Diabetes can cause various complications, one of which is microangiopathy, which is damage to the vascularization of the eye. Soyghurt is a probiotic drink high in isoflavones, has preventive benefits, and can be a supplement for patients with diabetes. This study aimed to determine the vascularization picture of the eye in alloxan-induced diabetic rats and given freeze-dried soyghurt. This study is a descriptive research using Posttest Only Control Group Design. The subjects of this study were white rats (*Rattus norvegicus*) Wistar strain, which were randomly separated into five groups, namely, three treatment groups and two control groups. Data were analyzed descriptively by describing eye vascularization assessment based on the formation of microaneurysms, hard exudates, cotton-wool spots, dot-

blot & flame hemorrhages, and neovascularization. The data processing results showed that the treatment group given a combination of freeze-dried soyghurt and metformin had the highest reduction in sugar levels in diabetic rats, with an average difference of 221.5 mg/dl. The administration of alloxan at a dose of 125 mg/kg of body weight for one day did not change the histopathological picture of eye vascularization in diabetic rats. Further tests need to be conducted regarding the appropriate dose and duration of alloxan administration to see the histopathological picture of eye vascularization damage.

**Keywords:** *alloxan; diabetes mellitus; eye vascularity; freeze-dried soyghurt*

#### **ABSTRAK**

*Diabetes mellitus adalah penyakit metabolik yang dapat menyebabkan hiperglikemia (peningkatan kadar gula darah). Diabetes dapat menyebabkan berbagai komplikasi, salah satunya adalah mikroangiopati, yaitu kerusakan pada vaskularisasi mata. Soyghurt adalah minuman probiotik yang tinggi isoflavon, dan memiliki manfaat pencegahan serta dapat menjadi suplemen untuk penderita diabetes. Tujuan dari penelitian ini adalah untuk mengetahui gambaran vaskularisasi mata pada tikus diabetes yang diinduksi alloxan dan diberi soyghurt beku kering. Penelitian ini merupakan penelitian deskriptif dengan menggunakan desain penelitian Posttest Only Control Group Design. Subjek penelitian ini adalah tikus putih (*Rattus norvegicus*) galur Wistar yang secara acak dipisahkan menjadi 5 kelompok, yaitu 3 kelompok perlakuan dan 2 kelompok kontrol. Data dianalisis secara deskriptif dengan menggambarkan vaskularisasi mata, yang dinilai berdasarkan pembentukan mikroaneurisma, eksudat keras, cotton-wool spots, dot-blot & flame hemorrhages, dan neovaskularisasi. Hasil pengolahan data menunjukkan bahwa kelompok perlakuan yang diberikan kombinasi soyghurt beku kering dan metformin memiliki penurunan kadar gula tertinggi pada tikus diabetes,*

*dengan perbedaan rata-rata 221,5 mg/dl. Pemberian alloxan pada dosis 125 mg / kg berat badan selama satu hari tidak memberikan perubahan dalam gambaran histopatologi vaskularisasi mata pada tikus diabetes. Tes lebih lanjut perlu dilakukan mengenai dosis dan durasi pemberian alloxan yang tepat untuk melihat gambaran histopatologi kerusakan vaskularisasi mata.*

***Kata kunci:*** *alloxan; diabetes mellitus; soyghurt beku kering; vaskularisasi mata*

## **INTRODUCTION**

Microangiopathy is a long-term vascular complication that is often found. Defects in these small blood vessels can cause damage to many tissues, especially in the eyes.<sup>1</sup> Damage to the retina due to diabetic microangiopathy is called diabetic retinopathy.<sup>2</sup> To prevent complications of DM, regular lifelong treatment is needed for maintaining blood sugar stability. Probiotic and prebiotic supplements are used as alternatives to prevent side effects.<sup>3</sup>

The ability of probiotics to lower oxidative stress, inflammatory response, and glucose levels in the periphery can prevent the onset and complications of DM. Probiotics can modulate immune and inflammatory responses by reducing cytokine activation and suppressing NF-K activity in the B pathway and also improving the intestinal microflora and maintaining intestinal permeability.<sup>4</sup> This

can prevent insulin resistance by reducing inflammation and improving pancreatic beta cells so that blood sugar levels can be well-controlled.<sup>5</sup>

In this study, we used probiotic bacteria on soyghurt that had undergone a freeze-dried process. Soyghurt is a probiotic drink made from soybean milk. Soybeans contain high levels of isoflavones and are very useful as antioxidants.<sup>6</sup> In this study, the probiotic bacteria used are *L. Acidophilus* and *B. Bifidum* are the dominant probiotic bacteria in the digestive tract and are proven to be very beneficial in maintaining health.<sup>7</sup>

## **METHOD**

This research has received approval from the Faculty of Medicine Ethics Commission, Unjani, no. 020/UHI.09.2023. This study is descriptive research using a Posttest Only Control

Group design. The research subjects were experimental animals that were randomly separated into five groups, each consisting of 5 rats. The subject of this study is rats (*Rattus norvegicus*). The freeze-dried soyghurt is soy milk fermented by *L. acidophilus* ATCC 4356 and *B. bifidum* ATCC 29521 with 3% inulin and 10% sucrose.

Male rats of the Wistar strain that had been divided into five treatment groups were adapted for seven days at the Animal Laboratory of Jenderal Achmad Yani Cimahi University consisting of K(-), K(+), P(1), P(2), and P(3). K(-) is a negative control that is only given standard feed and drink, K(+), P(1), P(2), and P(3) are a group of diabetic rats who are induced with alloxan 25 mg/rat intra-peritoneally once on day eight and then measured blood sugar levels periodically for up to 72 hours until the blood sugar level is >200 mg/dl.<sup>8</sup> On the next day, the P(1) group was given a treatment in the form of 9 mg/rat metformin, P(2) was given a treatment in the form of 0.2 ccs dry frozen soyghurt and P(3) was given a treatment in the form of a combination of 0.2 cc freeze dried soyghurt and metformin 9 mg/rat.<sup>9</sup> All treatment groups are given once a day, and they are given standard food and drink.

Before being induced with alloxan, all mice were checked for blood sugar levels

using the strip test method. Blood sampling for blood sugar level measurement was carried out three times, after the rats underwent the acclimatization period and, after being induced with alloxan precisely on the 10<sup>th</sup> day and after being treated for two weeks. Blood samples were taken from the tails of mice at as much as 0.5ml. Their blood sugar level was checked using a spectrophotometer.<sup>10</sup>

After two weeks of treatment, the mice were euthanized with CO<sub>2</sub> inhalation, and then surgery was carried out to remove the eye organs. The retrieved eye organ was part of the posterior wall. The eye organs were washed with 0.9% NaFis and 10% Neutral Buffer to remove the remaining blood.<sup>11</sup> The entire ocular bulb was made into a histopathological preparation with a coronal cut 4 microns thick, then stained with hematoxylin-eosin<sup>12</sup>, and the retinal blood vessels were assessed at the Laboratory of Anatomical Pathology, Jenderal Achmad Yani University. The preparation is read and validated by an anatomical pathologist.

The preparations were analyzed descriptively, describing ocular vascularization between treatment groups, and assessed based on the formation of microaneurism, hard exudates, cotton-wool spots, dot-blot & flame hemorrhages, and neovascularization.<sup>13</sup>

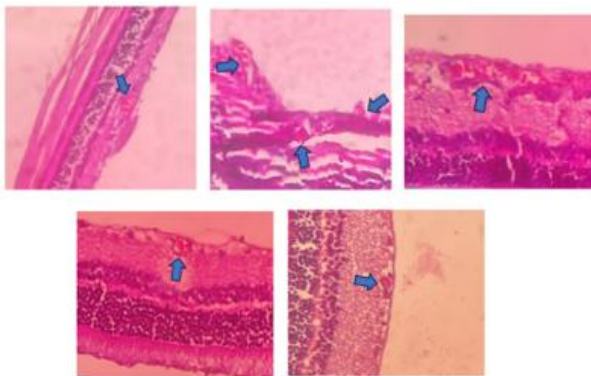
## RESULT

Blood sugar levels before and after treatment for 14 days in each group can be illustrated in the table.

**Table 1.** Comparison of Blood Sugar Levels of Diabetic Rats Before and After Treatment

No	Treatment	Before	After	Difference
1	Control (-)	85.26	83.3	1.96
2	Control (+)	323.8	238	85.8
3	Metformin	278	123.8	154.1
4	Freeze-dried soyghurt	345.6	177.8	167.8
5	Freeze-dried soyghurt and metformin	326.8	93.9	232.9

Based on Table 1, the group that showed the slightest difference (1,96 mg/dl) in blood sugar levels was the negative control group. In comparison, the group that showed the highest (232.9 mg/dl) difference in blood sugar levels was the group that combined soyghurt and metformin.



**Figure 2.** Histopathological picture of ocular vascularization after 14 days of treatment in each group. Arrows indicate normal blood vessels.

The description of the preparation in the five treatment groups was a 400x

magnification with hematoxylin-eosin staining, showing no abnormality in ocular vascularization, as seen in Figure 1.

## DISCUSSION

Table 1 of the negative control group, which was only given standard feed and drink, had the smallest average difference in blood sugar reduction compared to other groups. The decrease in blood sugar was likely due to the absence of damage to pancreatic beta cells so that insulin could work normally.<sup>14</sup>

In a combination group of freeze-dried soyghurt and metformin with standard feed and drink, the average difference in sugar content reduction was the highest. It is because soyghurt contains isoflavones that can reduce oxidative stress due to alloxan induction by inhibiting glucose oxidation and regenerating pancreatic cells.<sup>15</sup> In addition, probiotic bacteria modulate immune and inflammatory responses by reducing cytokine activation and suppressing NF-K activity in the B pathway, activating the immune system through toll-like receptor-4 (TLR-4). Probiotics also improve intestinal microflora and maintain intestinal permeability to prevent insulin resistance by reducing inflammation and improving pancreatic beta cells so blood sugar levels can be well-controlled.<sup>16</sup> It is

complementary to metformin, which decreases glucose formation in the liver, improves peripheral glucose uptake, and improves insulin sensitivity.<sup>17</sup>

Research conducted by Nawangsih, EN. et al. in 2022 proved that soyghurt significantly reduced blood sugar levels in alloxan-induced diabetic rats. The study also demonstrated that combining soyghurt and glibenclamide was better than giving exclusively glibenclamide or soyghurt.<sup>18</sup>

The description of the preparation in the five treatment groups showed no abnormalities in ocular vascularization. The vascular picture is characterized by a collection of bright red erythrocytes encased by endothelial membranes on the internal limitans membrane of the retina.<sup>19</sup> Figure 1 shows no compensated microaneurysms of the precapillary arterioles, no thickening of the basal membrane of the retinal capillaries, and the absence of microaneurysms, hard exudates, cotton-wool spots, dot-blot & flame hemorrhages, and neovascularization.<sup>20</sup>

Alloxan-induced diabetes in rats primarily causes damage to the pancreatic beta cells, leading to insulin deficiency and hyperglycemia.<sup>21</sup> However, the absence of vascular abnormalities in the eyes of these rats after 14 days may be due to several reasons:

1. Duration of induction: The 14-day

period may be too short to observe significant microvascular changes in the retina. Diabetic retinopathy, which involves vascular abnormalities, usually develops over a more extended period as a chronic complication of diabetes.<sup>22</sup>

2. Model specificity: The alloxan model of diabetes, while effective in inducing hyperglycemia, might not perfectly replicate all the complications seen in human diabetes, such as microvascular damage in the retina.<sup>23</sup>

3. Vascular resilience: In some cases, the vascular system of rats may initially withstand hyperglycemia without immediate visible histopathological changes, especially if the duration of hyperglycemia is relatively short.<sup>24</sup>

## CONCLUSION

This study showed a significant difference in blood sugar levels between the positive control group (DM rats without therapy) and the DM rat group that was given soyghurt alone or the group that was given a combination of soyghurt and metformin. However, no abnormalities in the vascularization of the eyes were found. Further research is needed, with a longer

duration of treatment, to get an overview of complications in the ocular vascular system.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article. All research was conducted independently and impartially.

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