

## Research article

# Effect of Soyghurt *Lactobacillus Acidophilus* on Blood Glucose Levels in Alloxan-Induced Diabetic Rats

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Management of diabetes melitus is still a global problem because satisfactory therapeutic treatments have not been found. This study aimed to explore the possibility of soyghurt *Lactobacillus (L.) acidophilus* as an alternative therapy to lower blood glucose levels through the administration of soyghurt *L. acidophilus* to alloxan-induced diabetic rats. This was experimental laboratory research with a pre- and post-test design and control group. It was found that administering soyghurt *L. acidophilus* had a significant influence, but a combination of soyghurt *L. acidophilus* and glibenclamide was the most effective in lowering the blood glucose levels of the diabetic rats ( $p < 0.05$ ). The blood glucose levels were reduced due to the local and systemic effects of soyghurt *L. acidophilus* in reducing proinflammatory cytokines. This complemented the mechanism of action of glibenclamide which increases insulin secretion. We can conclude that a combination of soyghurt *L. acidophilus* and glycbenklamid results in the largest decrease in blood glucose levels.

**Keywords:** blood glucose, diabetic rat, *L. acidophilus*, soyghurtCorresponding Author: Eka  
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## 1. Introduction

The global diabetes melitus (DM) prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.[1] With the increase in DM patients, there is also an increase in morbidity and mortality due to the complications it causes.[2] In addition, therapeutic side effects are increasing, therefore it is necessary alternative therapy for patients with DM.

One of the DM alternative therapies developed is probiotic therapy. In a study conducted by Albeer El Sayed in 2010, stated that Bifidobacterium and *L.acidophilus* can lower the blood glucose levels of diabetic rat.[3] Similarly, research conducted by Nawangsih EN in 2017, can prove a decrease in blood glucose levels of diabetic rat after administration of Munghurt *L.acidophilus*.[4]

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Most probiotics are consumed by the public, using cow's milk media for its growth.[5] Protein content in cow's milk can cause allergies in hypersensitive people,[6] therefore another alternative medium is needed for *L.acidophilus* growth media. The study used soy beans (glycine max) as a medium for the growth of probiotic bacteria. Soyghurt is a product of fermented soy milk using probiotic bacteria.[7] Soy protein has several advantages, among others: its essential amino acid content is highest when compared to other nuts and its fat content is unsaturated fatty acids. In addition, soy protein is hypocholesterolemic and hypoglycemic [4]. Although soy milk does not contain lactose as in cow's milk, lactic acid bacteria can use natural carbohydrate sources in soybeans such as sucrose, raffinose, and stachyose as its energy source.[8]

The administration of *L.acidophilus* bacteria found in soyghurt can decrease blood glucose levels due to increased normal flora and intestinal permeability. Increased intestinal permeability can decrease proinflammatory cytokines that can damage the pancreas. In addition, the advantage of this study is the use of soy milk as medium for the growth of *L.acidophilus*. Soy beans contain flavonoids that can reduce oxidative stress so that it can minimize and repair pancreatic damage. [8]

Based on the above description, researchers are interested in examining the influence of soyghurt on the blood glucose levels of *alloxan*-induced rat. In this study, soyghurt *L.acidophilus* was used orally in diabetic rat compared to anti-diabetic drugs, namely *glibenclamide* and how it would affect when the drug was combined with soyghurt *L. Acidophilus*.

## 2. Methods

This study using *L. Acidophilus* ATCC 4356 and soybeans bromo varieties. Tryptone Soya Broth (TSB) medium for rejuvenation of bacteria and *De Man Rogosa Sharpe agar* (MSA) medium for bacterial growth medium.

### 2.1. Bacterial Re-identification

The purpose of bacterial re-identification is to ensure that the bacteria used are actually *L. acidophilus* and not contaminants. The re-identification methods used in this study consist of macroscopic test, microscopic test, and biochemistry test. Macroscopic test aims to identify the typical colony of the bacteria. Microscopic test aims to observe Gram staining results, bacterial morphology and formation. Biochemistry tests to identify bacteria's ability to produce catalase and to ferment carbohydrates.[9]

## 2.2. Preparation of soy milk

Wash soy beans until clean. Soy beans is boiled for about 15 minutes, then soaked in clean water for 12 hours. Soy beans are washed until the skin peels off, then crushed with a blender until crushed. Crushed soybeans are mixed with hot water and stirred until well. After that, it is filtered with a clean cloth until a solution of soy milk is obtained.. Sterilization process continued for 15 minutes in an autoclave at a temperature 121°C and two atm pressure.[10]

## 2.3. Manufacture of Bacterial Starter

Colonies of re-identified and rejuvenated bacteria are suspended by mixing *L.acidophilus* colonies with 0.9% NaCL until Mc farland's turbidity level is equivalent to  $10^7$ CFU/mL. The turbidity level of this starter solution is measured using spectrophotometer.[10]

## 2.4. Fermentation of Soy Beans Milk

Soy bean milk as much as 9 ml plus 1 ml of starter suspension. The mixture is homogenized using a vortex, then fermented for 48 hours at a temperature of 37<sup>0</sup>C inside the incubator. After that, the number of bacterial colonies is calculated by the TPC method to obtain the amount of bacteria in the probiotic before treatment.[10]

## 2.5. Bacterial Amount Counting

Identified and rejuvenated bacterial colonies are made suspense by mixing *L.acidophilus* colonies with NaCL 0.9% until they get a degree of McFarland's turbidity equivalent to  $10^7$ CFU/mL using a spectrophotometer.[9]

## 2.6. Experimental Animals

A total of 25 wistar rat were divided into 5 groups. Negative control of K(-) was only given standard feed and drinking without *alloxan*-induced, while positive controls K(+), K1 ,K2 and K3 were groups of rat induced by *alloxan* to become diabetic rat. The rat were injected intraperitoneally at a dose of 120 mg/kgBB daily from day 8 to day 12 and the last three days were given a 10% glucose mixture. Treatment began on the 13th day. Probiotics and glycbenklamid are administered orally at doses of  $10^7$  CFU/ml and 0.45

mg/kgBB. Treatment is carried out daily for 4 weeks. The five treatment groups are as follows:

1. K (-): negative control group, fed and drinking standard
2. K (+): positive control group, induced acoxon and given standard feed and drinking.
3. K1: this group is induced *alloxan*, given standard feed and drinking as well as soyghurt *L.acidophilus*
4. K2: this group is *alloxan* induced, given standard feed and drinking as well as *glibenclamide*
5. K3: this group is induced *alloxan*, given standard feed and drinking as well as a combination of soyghurt *L.acidophilus* and *glibenclamide*.

## 2.7. Blood Collection and Blood Glucose Level Measurement

Blood glucose levels were measured twice, i.e. after induced *alloxan* on the 13th day and after completion of treatment for 4 weeks.[4] Blood is taken from the arteries of the eye vessels (sinus orbitalis) of *rat* as much as 0.5ml and accommodated in microhemathic tubes.[11] After that it is centrifus for 15-20 minutes at a speed of 3000 rpm to obtain blood plasma. The blood glucose level test uses a spectrophotometer with the GOD-PAP method at a wavelength of 546 nm.[12]

## 2.8. Statistical Analysis

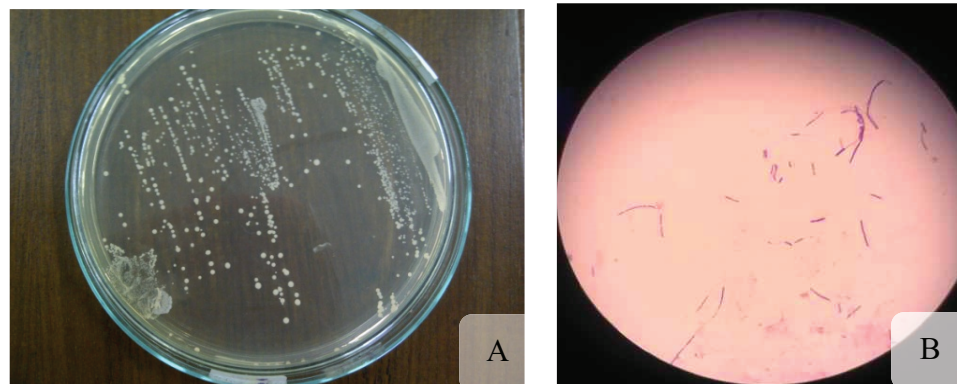
The data were analyzed with a paired T test to determine the effect of each treatment on the blood glucose levels of diabetic rat. As for knowing which treatment group was best at lowering the blood glucose levels of diabetic, researchers continued to analyze the data with the Man Whitney test. p values of <0.05 were considered to be significantly different.[13]

# 3. Results

## 3.1. Bacterial reidentification

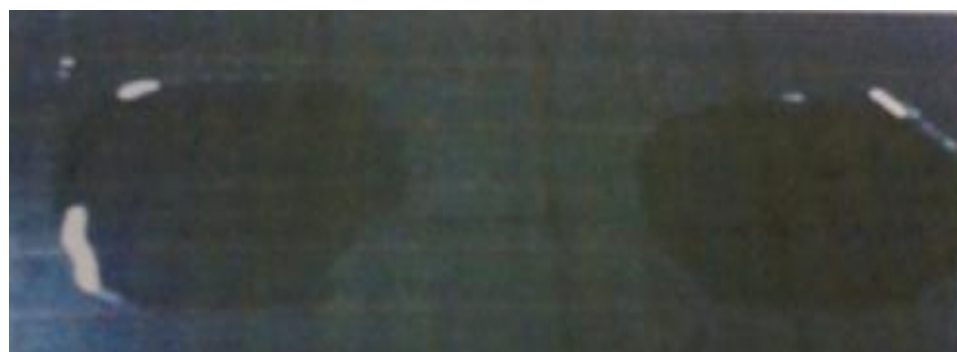
Result of macroscopic examination on MRS medium: white colony, pinpoint, diameter of colony 0,5-1,5 mm, convex elevation, smooth-edged and grayish-white color.

Aroma resulting from bacterial colonies was sour aroma. This is in accordance with the parameter of characteristics Lactobacillus colony morphology. Results of microscopic examination: Gram positive rod, chain formation. The results of this examination in accordance with microscopic characteristic of *L. acidophilus*. [9]

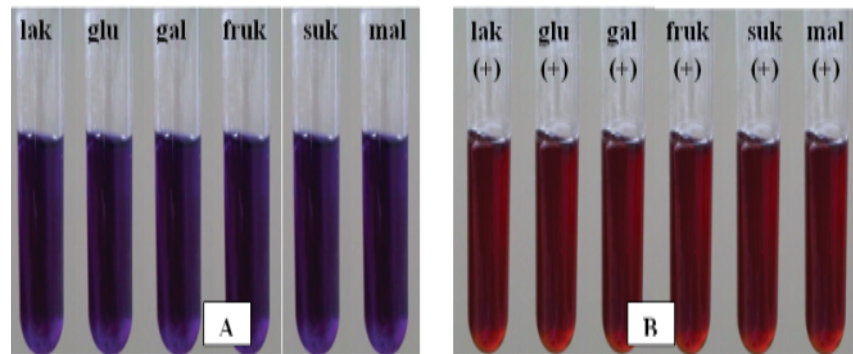


**Figure 1:** Results macroscopic and microscopic examination of *L. acidophilus* A: colony morphology B: result of Gram staining.

The results of biochemistry examination for catalase test showed negative results. Catalase test aims to identify the bacteria's ability to transform  $H_2O_2$  into  $H_2O$  and  $O_2$  using the enzyme catalase. The results obtained in the catalase test is negative, characterized by no formation of oxygen bubbles because Lactobacillus acidophilus does not produce the enzyme catalase to convert hydrogen peroxide ( $H_2O_2$ ) into water ( $H_2O$ ) and oxygen ( $O_2$ ). Catalase test results according to parameters. The results of biochemistry test for carbohydrate fermentation were positive fermentation for lactose, glucose, galactose, fructose, sucrose, and maltose. Using brom cresol purple (BCP) as an indicator, positive results are marked with purple changes to red color. It is caused the test bacteria produced lactic acid which can lower the pH became acidic. thus it can be concluded that all of the results suitable for *L. Acidophilus* without contamination. [14]



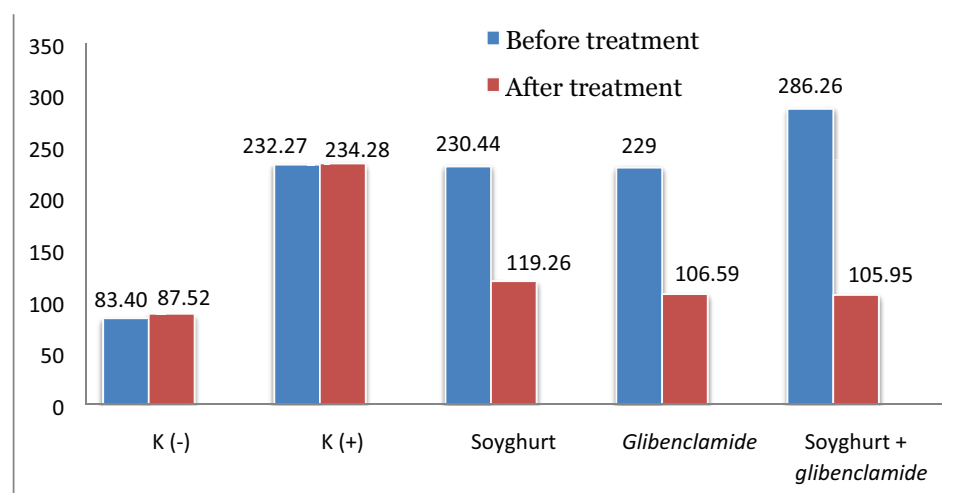
**Figure 2:** Negative result of catalase Test.



**Figure 3:** (A) Before fermentation (B) Positive result for Carbohydrate fermentation by *L. acidophilus*.

### 3.2. Blood Glucose Levels Before and After Treatment

The study analyzed blood glucose levels in the control group and treatment group, and observed which treatment was best at lowering blood glucose levels. The following are blood glucose levels in Wistar rat, before and after treatment for 28 days. Based on Figure 1, blood glucose levels in negative control (K-) are relatively normal, while in positive control (K+) there is an increase in blood glucose levels of more than 200 mg/dl. Thus, K(-) and K(+) qualify as controls.



**Figure 4:** Difference in average blood glucose levels before and after treatment.

The data on table 1, further tested with a T-pair test to determine the effect of treatment on the blood glucose levels of Wistar rat by analyzing differences in blood glucose levels before and after treatment.

Meanwhile, to find out which differences between groups and which groups were best at lowering the blood glucose levels of diabetic rat, the Man Whitney test was conducted.

TABLE 1: Analysis of blood glucose levels before and after treatment.

No	Treatment group	Average blood glucose levels before treatment (mg/dl) ±SD	Average blood glucose levels after treatment (mg/dl) ±SD	Difference (mg/dl)	P <sup>*)</sup>
1	control (-)	83,40±5,195	87,52±4,857	-4,123	0,00
2	control (K+)	232,22±17,079	234,28±16,830	-2,010	0,00
3	K1 (Soyghurt)	230,44±26,114	119,26±7,309	111,178	0,00
4	K2 (Glibenclamide)	229,00±12,819	106,59±3,137	122,416	0,00
5	K3 (Soyghurt+Glibenclamide)	286,26±13,904	105,95±5,432	180,314	0,00

P<sup>\*)</sup>= base on t-test

Description:

Control (-) the group without alloxan induction, standard meals.

Control (+) alloxan induction group, standard meals.

K1 alloxan induction group, soyghurt treatment, standard meals

K2 alloxan induction group, glibenclamide treatment, standard meals

k3 alloxan induction group, glibenclamide+soyghurt treatment, standard meals

TABLE 2: Analysis of blood glucose levels in each treatment group.

Kelompok	Kelompok pembanding	P <sup>*)</sup>	Description
Control (-)	Control (+)	0,009	Significan
	KP1 (Soyghurt)	0,009	Significan
	KP2 (Glibenclamide)	0,009	Significan
	KP3(Soyghurt+Glibenclamide)	0,009	Significan
Control (+)	Control (-)	0,009	Significan
	KP1 (Soyghurt)	0,009	Significan
	KP2 (Glibenclamide)	0,009	Significan
	KP3(Soyghurt+Glibenclamide)	0,009	Significan
KP1 (Soyghurt)	KP2 (Glibenclamide)	0,251	Tidak signifikan
	KP3(Soyghurt+Glibenclamide)	0,009	Significan
KP2(Glibenclamide)	KP3(Soyghurt+Glibenclamide)	0,009	Significan

P<sup>\*)</sup>= base on Man Whitney test

## 4. Discussion

In Table 1 obtained results, the control group was negative, the average blood glucose was normal before and after the 28-day treatment, which was 83±5,195 mg/dL and 87.52±4,857 mg/dL. In addition, blood glucose levels increased by 4,123 mg/dL. This group was not *alloxan*-induced and given standard meals. The increase in the blood glucose levels of *rat* in negative control group was likely due to the glucose content found in the mouse feed. The blood glucose levels of *rat* in this group were still within normal limits so could still be used as a reference for normal blood glucose levels. Blood glucose levels that do not increase significantly are likely caused by pancreatic

cells that are still working and produce insulin normally. Pancreatic cells do not suffer damage from induction by *alloxan* as occurs in other induced treatment groups.[15]

In the positive control group, blood glucose levels before and after the 28-day treatment were high, namely  $232 \pm 22 \pm 17,079$  mg/dL and  $234 \pm 28 \pm 16,830$  mg/dL. In addition, there was also an increase in blood glucose levels of 2,010 mg/dL. This group was *alloxan*-induced and given standard meals. High blood glucose levels are caused by the mechanism of action of *alloxan* that reacts by damaging the essential substance in pancreatic beta cells resulting in reduced insulin-carrying granules in pancreatic beta cells.[15,16] Toxic action of *alloxan* in beta cells is initiated by free radicals formed by redox reactions.[15] Free radicals with high stimulation increase the concentration of cytosol calcium which causes rapid destructiveness of pancreatic beta cells, resulting in increased glucose levels in the blood. This increased blood glucose level is what proves that *alloxan* can increase the blood glucose of Wistar *rat*. [16]

In all treatment groups (K1-K3) there was a significant decrease in blood glucose levels. To find out which treatment group was best at lowering the blood glucose levels of the *rat*, the researchers continued to analyze the data with the Man Whitney test. In Table 4.2 based on the Man Whitney test, there were insignificant results with a value of  $p=0.251$  ( $p>0.05$ ) in the soyghurt treatment group compared to the glibenclamid treatment group. This suggests that between *glibenclamide* and soyghurt statistically has the same effect in lowering the blood glucose levels of *rat*. Nevertheless, significant results were found with a value of  $p=0.009$  ( $p<0.05$ ) in the soyghurt (K1) and *glibenclamide* (K2) treatment groups with a combination treatment group of soyghurt and *glibenclamide* (K3).. This showed that the administration of a combination of soyghurt and glycbenklamid was better at lowering the blood glucose levels of *rat* dialytic when compared to the administration of *glibenclamide* or soyghurt alone. The decrease in glucose levels in this combination group is likely due to the effect of glycbenklamid and soyghurt on complementary pancreatic beta cells. The mechanism of action of glycbenklamid is to increase insulin secretion from pancreatic beta cell granules so that glucose in the blood can decrease due to increased insulin secretion.[17] Meanwhile, the decrease in blood glucose in soyghurt is caused by local and systemic effects. The local mechanism of action in soyghurt is caused by the work of the E-cadherin/Beta-cetatin complex which can improve intestinal permeability. In addition, there is also an increase in the number of normal microflora of the intestine that can increase the number of panet cells. These cells produce antimicrobial proteins as a first-line defense against pathogens and play a role in improving intestinal permeability.[15,18] Improved intestinal permeability will have systemic effects, namely suppressing inflammatory processes by



inhibiting proinflammatory cytokines that induce damage to beta cells so as to minimize damage to beta pancreatic cells. In addition, the decrease in blood glucose can also be caused by the content of flavonoids in soybeans which has the effect of lowering oxidative stress by bonding with free radicals caused by the induction of *alloxan*.<sup>[17]</sup>

The limitation of this study is the absence of pancreatic histopathology examination in experimental rat to confirm the presence of pancreatic damage after induction of *alloxan*.

## 5. Conclusion

This study proves that soyghurt had a very significant effect on the decrease in blood sugar levels of diabetic rat. The combination of soyghurt and *glibenclamide* was better at lowering the blood sugar levels of diabetic rat when compared to *glibenclamide* or soyghurt alone. More research is needed with human research subjects, so that soyghurt *L. acidophilus* can be a supplement therapy for diabetics.

## Conflict of Interest

There was no conflict of interest in this study

## Acknowledgment

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