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# Identification of lactic acid bacteria from lemea's to lower blood sugar levels in mice's diabetes mellitus

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**ABSTRACT**

**Background**: Diabetic is one of the main health problems in society throughout the world, so alternatives are needed to help prevent increasing blood sugar levels. In this case, lactic acid bacteria (LAB) are able to inhibit the performance of the alpha glucosidase enzyme in breaking down carbohydrates into glucose, lowering blood sugar levels so that lactic acid bacteria have potential in controlling hyperglycemia.

**Objectives**: The purpose of this work is to assess the potential of four different strains of Lactobacillus that were isolated from lemea to reduce blood sugar levels in mice.

**Materials and Methods**: This study used a pre-test, post-test, control group design for its experimental methodology. Six groups of 18 male mice, aged between 10 and 12 weeks, with body weights ± 25 g each, were formed,three mice comprised each group. This study was divided into 6 treatment groups, namely 1 group given acarboce, 1 group without treatment (aquadest), and 4 treatment groups given 4 types of pure BAL culture. Treatment consisted of P1 (giving L plantarum B1), P2 (giving L plantarum B2), P3 (giving L plantarum S1), and P4 (giving L fermentum S2). The initial stage of the research was to measure the initial fasting blood sugar of the mice, then alloxan was induced at a dose of 4.54 mg/kg BW and given intravenously at 0.1/10 g BW to the mice. After 5 days of alloxan induction, the fasting blood sugar level was measured again. If the blood sugar level was ≥ 127 mg/dL, the mice were declared DM and ready to be given treatment. The dose of acarbose was 0.65 mg/kg bw, and BAL culture with a concentration of 10 $^{8}$ CFU/mL of 0.1 mg/ 10 g bw for 14 days every 7 days analyzed the blood sugar levels of the mice.

**Results**: The results of the study showed that giving pure culture of L. plantarum from Betung bamboo shoot lemea to mice with type 2 diabetes mellitus (type 2 DM) as treatment P2 was superior to giving acarbose, P1, P3, and P4 in reducing blood sugar levels with pancreatic damage 50% and lower than other treatments, while the pancreas of type 2 DM mice without therapy had the greatest damage score based on histopathological data.

**Conclusion**: Lactic acid bacteria from Lemea can help lower blood sugar levels and prevent the rate of damage to the pancreas of diabetic mice, with the best culture being L. plantarum from Lemea Betung bamboo shoots.

***Keywords*** *: blood sugar levels; lactic acid bacteria; lemea; pancreatic*

## BACKGROUND

Diabetes is an endocrine system disorder characterized by elevated blood glucose levels.[1] The primary symptom of diverse metabolic problems that lead to diabetes mellitus (DM) is persistent hyperglycemia, which is brought on by abnormalities in insulin production or insulin resistance, or even both.[2] DM poses a major risk to world health. Over the past thirty years, the global population of those affected has quadrupled.[3] 693 million adults worldwide will be attacked by DM in 2045; this is a greater than 50% increase from 2017.[1] Type 2 diabetes is becoming more common in both industrialized and developing nations, and obesity rates are rising at the same time.[3]

LAB may transform the carbohydrates found in fruits and vegetables into lactic acid, which is marked by a pH drop to 4.0 in the fermentation product. Products fermented from bamboo shoots can include LAB.[4] Lemea is a fermented product made by the Rejang people in Bengkulu, Indonesia which were retrieved from bamboo shoots.[5] Fermented bamboo shoots that has health, in India referred to as “green gold”.[6] According to the findings of ethnobotanical study, bamboo plants are utilized in Indonesian traditional medicine.[7,8]

LAB can modulate gut microbiota, which plays an important role in liver health. Strains such as *Lactobacillus plantarum* and *Lactobacillus acidophilus* have been shown to reshape the gut microbiota, increasing beneficial bacteria and short-chain fatty acid (SCFA) producers, which in turn improves liver function.[9] *Lactobacillus plantarum* SHY130 has been shown to improve liver function and regulate hepatic metabolism in diabetic mice, involving pathways like purine metabolism and amino acid metabolism.[10]

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Therefore, the purpose of this study was to ascertain whether giving lactic acid bacteria to DM mice (*Mus musculus*) would lower blood sugar levels and prevent histological damage to the pancreas. Lactic acid bacteria from Lemeas's can reduce Blood Sugar Levels in mice.

## MATERIALS AND METHODS

### Sample Preparation

This study uses an experimental design with a pre-test, post-test, and control group. Six groups of 18 male mice, aged between 10 and 12 weeks, with body weights ± 25 g each, were formed. Three mice comprised each group. The following is how each group treated:

Acarbose is the control (+)
Control / Absence of therapy (-)
P1: *Lactobasilus plantarum* B1 culture administration
P2: *Lactobasilus plantarum* B2 culture administration
P3: *Lactobasilus plantarum* S1 culture administration
P4: *Lactobasilus fermentum* S2 culture administration

Lactic acid bacteria culture administration include: P1 is (*Lactobasilus plantarum* strain from Lemea Betung bamboo shoots 1), P2 (*Lactobasilus plantarum* strain from Lemea Betung bamboo shoots 2), P3 (*Lactobasilus plantarum* strain from Lemea yellow bamboo shoots), and P4 (*Lactobasilus fermentum* strain from Lemea yellowbamboo shoots).

**Procedure of measuring blood glucose**Measurement of blood sugar levels**:** A digital scale was used to weigh male Swiss webster mice (Mus musculus) that were between 10 and 12 weeks old. Mice were fasted from food for 18 hours, and blood samples were taken from the injured tip of the *Glucometer One Touch Horizon so that within five seconds, the blood sugar levels of mice could be obtained*. The following formula can be used to determine the relative blood glucose level:

$$\text{Relative blood glucose level} = \frac{\text{blood glucose level at time t}}{\text{intial blood glucose level}} \text{ x } 100\%$$

**Induction of Alloxan in Mice**DM induction in mice treated with alloxan: An intravenous injection of 0.1 mL/10 g body weight and an alloxan treatment dose of 4.54 mg/kg body weight were administered to mice. The mice will be housed in cages and fed food while alloxan is administered. If the blood sugar levels is ≥127 mg/dL, it is classified as diabetes mellitus and treatment can be initiated. The blood sugar levels is remeasured and examined after 5 days.

**Lactic acid bacteria treatment** for two weeks, mice with blood sugar levels ≥127 mg/dL will receive a dose of pure BAL culture suspension at a concentration of $10^8$ CFU/mL 0.1 mL/10 g BW of mice. Acarbose was given to the control (+) group at a dose of 0.1 mg/10 g body weight or 0.65 mg/kg body weight of mice, whereas no treatment was provided to the control (-) group. A blood sugar levels assay is performed every seventh day. The lactic acid bacteria were prepared 48 hours in advance of the mice's administration. The first step in preparing to deliver lactic acid bacteria is to take three to five colonies of MRSA-purified LAB isolates, put them in a test tube with 5 mL of NaCl, and use a vortex to homogenize the mixture.

**Procedure of histopathological analysis**

After receiving treatment, the mice's final fasting blood sugar levels are recorded. The mice are then put to sleep so that the pancreas can be removed and the pancreatic histopathology can be examined under a 400x magnification microscope. The pancreas of each treated mouse is then scored from zero (0) to four (4). The following categories form the basis of histopathology scoring:

Score 0: Normal pancreatic histopathology, with no alterations to the islets of Langerhans' boundaries, cell count, necrotic cell content, or morphology.
Score 1: The pancreatic histopathology is 25% damaged, has distinct borders, the number of cells is beginning to decline, there is just cell degeneration at this time, and the shape of the cells is normal;
Score 2: The pancreatic histopathology exhibits 50% damage, ill-defined borders, a decrease in cell quantity, degeneration, and aberrant morphology of cells;
Score 3: The pancreas's histology shows 75% damage, hazy borders, fewer cells overall, obvious necrotic cells, and aberrant cell shapes in many of them;


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Score 4: There is 100% damage to the pancreatic histology, with ill-defined boundaries.

## RESULTS

### *Blood sugar levels*

Taking a mouse's blood at the tip of its tail to determine its blood sugar level using a glucometer. The mice's blood sugar was assessed on day 0 (D0) prior to the mice being classified as diabetic. The first day that mice are diagnosed with diabetes and begin treatment is known as D1. D2 blood sugar levels following a seven-day therapy intervention. D3 blood sugar level after 14 days. Table 1 displays the blood sugar levels after a fast.

**Table 1. Mice (*Mus musculus*) blood glucose levels for each treatment group**

| Treatment | Mean Blood Glucose Levels | | | |
|---|---|---|---|---|
| | DO | D1 | D2 | D3 |
| Acarboce (Control Positive) | 74.67±8.15 | 135.00±3.61 | 107.67±12.01 | 96.33±13.87 |
| Absence of therapy (Control Negative) | 88.67±9.07 | 141.33±16.29 | 123.33±5.03 | 118.33±5.51 |
| P1 | 84.33±18.01 | 140.00±13.89 | 103.00±2.00 | 98.33±16.26 |
| P2 | 78.67±14.74 | 157.00±8.72 | 125.00±1.73 | 105.33±7.77 |
| P3 | 85.67±15.95 | 146.00±17.35 | 107.33±8.96 | 107.67±42.77 |
| P4 | 82.67±8.02 | 145.67±29.77 | 108.67±16.77 | 111.33±17.21 |

D0: blood sugar level of diabetic mice before treatment
D1: The first day that mice are diagnosed with diabetes
D2: blood sugar levels after treatment for 7 days
D3: blood sugar levels after treatment for 14 days

The results of this study indicate that when mice were given acarboce and pure BAL culture, their fasting blood sugar levels decreased. In Figure 1, the relative mean blood sugar is displayed. After alloxan intervention, mice's fasting blood sugar levels increased to more than 127 mg/dL (D1), declined after 7 days of alloxan administration (D2), and remained stable until day 14 (D3). Relative blood sugar information was not statistically different ($p > 0.05$) with two-way ANOVA.

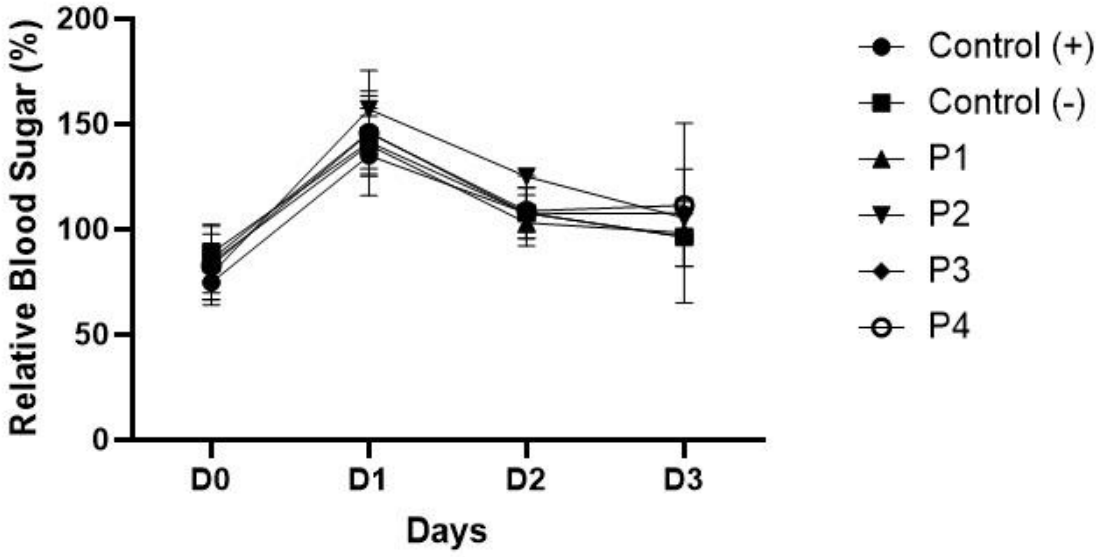


**Figure 1. Mean relative blood sugar profile for all groups receiving therapy**

The mice (*Mus musculus*) in the group that received the bacterial culture *Lactobacillus plantarum* B2 (P2) coming from Lemea with betung bamboo as raw material had the greatest drop in blood sugar levels, reaching


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to 51.67%. Conversely, diabetic mice receiving no therapy (negative control) had the lowest effectiveness, at 23.00%. Decrease in blood sugar information was not statistically different ($p > 0.05$) with one-way ANOVA. (Figure 2).

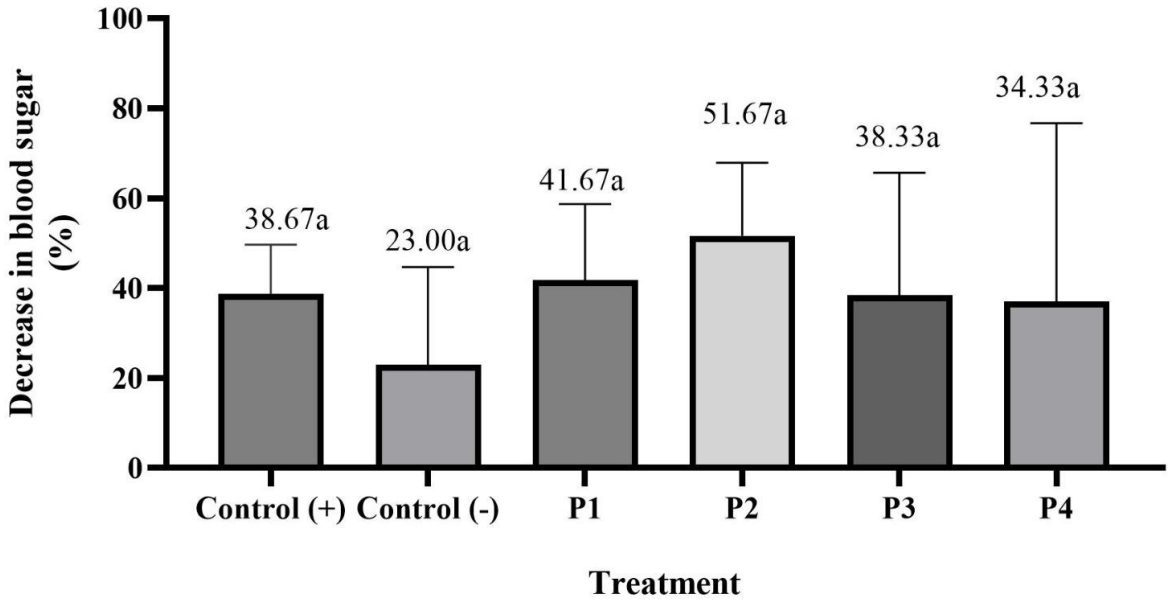


**Figure 2. Decreased blood sugar levels in six groups of mice**

***Pancreatic histopatology***

Following histological examinations, the mouse pancreas was assigned scores (0, 1, 2, 3, and 4). Figure 3 shows the outcomes for each treatment group.

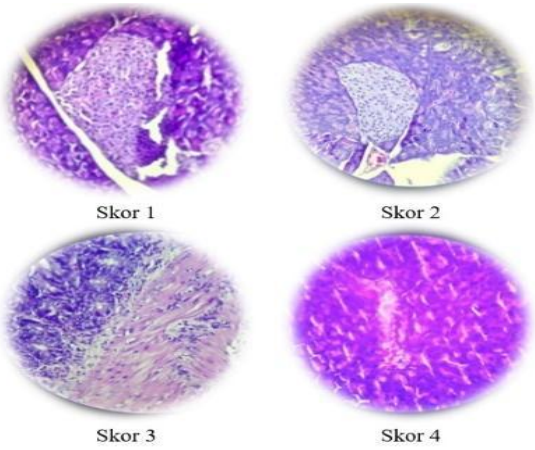


**Figure 3. 400x HE magnification of the mice pancreatic histopathology**

Images with a score of 1 are in pancreas P1; score 2 was obtained in the pancreas by administering acarbose; a score of 3 was obtained from pancreatic P3; and a score of 4 was obtained from the pancreas absence of therapy. Based on histological observations of the pancreas made with three viewing area repeats using an ocular microscope at HE 400x magnification, it was found that DM mice (*Mus musculus*) that were not given pure BAL culture suffered the greatest pancreatic damage. animals administered *Lactobacillus plantarum* B2 had the lowest pancreatic damage score ($2.00 \pm 0.00$), which indicates 25% damage. These animals showed distinct borders, a decrease in the number of cells, solely cell degeneration rather than necrosis, and normal cell shape (Table 2).


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**Table 2. Scores for Pancreatic Histopathology**

| Group | Pancreatic Damage Score | |
|---|---|---|
| | Average | SD |
| Absence of therapy | 4.00 | ±0.00 |
| *Acarbose* | 3.00 | ±1.00 |
| P1 | 2.00 | ±1.00 |
| P2 | 2.00 | ±0.00 |
| P3 | 2.00 | ±1.00 |
| P4 | 1.67 | ±0.58 |

Score for Pancreatic Histopthology information was not statistically different ($p > 0.05$) with one-way ANOVA

## DISCUSSION

The results of this study found that diabetic mice experienced a decline after 7 days of lactic acid bacteria culture intervention and tended to stabilize after 14 days. The highest percentage reduction was in mice given the intervention of *Lactobacillus plantarum* B2 isolated from lemea with the betung bamboo type. In line with research, ginseng berries fermented with Lactobacillus plantarum are known to lower blood sugar in mice.[10]

When pancreatic *β cells* are damaged in mice given alloxan, the result is decreased insulin secretion and elevated blood sugar levels. This leads to the development of diabetes in the mice.[11] Acarbose is an artificial oligosaccharide that functions as a competitive inhibitor, slowing down the digestion of carbohydrates and lowering blood sugar levels by obstructing the α-glucosidase enzyme's activity.[12] Unwanted drug reactions, often known as adverse drug reactions (ADRs), are one of the issues brought on by drug use. Acarbose is one of the oral hypoglycemic medications that causes ADRs, including a flatulence effect in as many as 8.1% of patients.[13]

Antioxidant activation is present in *L. plantarum* probiotics both in vitro.[14] The activation of α-glucosidase inhibitor, antioxidants, and antiglycation characteristics of lactic acid bacteria can lower blood sugar levels or even prevent diabetes.[15]

Furthermore, secondary metabolites from LAB have the ability to adhere to and colonize intestinal mucosa, which inhibits the growth of harmful bacteria. The gut microbiota's makeup will alter in people with type 2 diabetes. Probiotics have been shown in studies on laboratory animals to change the gut microbiota's composition by elevating the *Firmicutes phylum* and lowering the Bacteroidetes, which cause inflammation. Compared to the prior environment with larger cages, where the environment could alter blood sugar levels, mice were placed in small cages measuring 22 x 31 x 9.5 cm, which could raise stress and decrease physical activity.[16] Diabetes patients may experience elevated blood sugar levels as a result of stress and inactivity.[19,20,21]

Because they activate α-glucosidase inhibitors, lactic acid bacteria are useful in lowering blood sugar levels. the bacterial strain *L. paracasei* TD062 can regulate fasting blood glucose levels and play a significant role in avoiding the development of type 2 diabetes mellitus due to its high α-glucosidase inhibitory activity (31.9%) and good antidiabetic capacity. By controlling gut bacteria, *L. acidophilus* probiotics can ameliorate type 2 diabetes by controlling liver lipid metabolism and glucose levels.[20] Lactic acid bacteria (LAB) is a type 2 diabetes patient's gut microbial balance-restoring bacterium. Furthermore, diabetic mice's fasting blood glucose levels can be lowered by lactic acid bacteria (*Lactis plantarum*) isolated from kimchi, and type 2 diabetes mice under prolonged cold stress can have their pro-inflammatory activity suppressed.[21]

The diabetic mice were fed a regular pellet meal during treatment, which included 20% protein, 4% fat, 4% crude fiber for mice, and distilled water. This may have contributed to the drop in blood pressure in the negative control mice, offering banana peel biscuits with high crude fiber will assist lower the blood sugar levels of hyperglycemic male mice (*Mus musculus*). This suggests that the low carbohydrate content and crude fiber form of the food might help control blood sugar levels.

Pancreatic β cell dysfunction and cell death will impact the development of diabetes mellitus, including type 1 and type 2 DM. In type 2 diabetic, genetic signals play a major role in regulating the growth and function of β cells, allowing metabolic stress to hasten the loss of β cell mass.[22] By supplying lactic acid bacteria, which also have antioxidant qualities, pancreatic cell damage can be prevented. Treatment of nutmeg extract was able to increase the number of β cells in the pancreatic islets so that they could make insulin and help lower blood sugar levels. This is consistent with the observation that the number of β cells reduced in diabetic mice.


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## CONCLUSIONS

The *Lactobacillus plantarum* strain from betung bamboo shoots (B2) strain was found to be most effective in lowering blood sugar levels in diabetic mice. Mice (*Mus musculus*) fed *Lactobacillus plantarum* B2, a lemea strain that uses bamboo shoots (*Dendrocalamus asper* (Schult. & Schult.f) Backer) as a raw material, showed 50% damage to the pancreas. although statistically it has no effect.

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