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Research Article
Effect of Munghurt *Lactobacillus acidophilus* from Green Beans to Blood Glucose Levels in Alloxan-induced Diabetic Rats

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Abstract
Background and Objective: Munghurt is a probiotic made from green beans. Mung beans possess enzymes, nutrients, antioxidants and low fats essential for health. Green beans contain higher carbohydrate than cow milk better to growth *Lactobacillus acidophilus*. The objective of this research was to determine effect of munghurt *Lactobacillus acidophilus* to blood glucose levels in diabetic rats induced alloxan. **Materials and Methods:** The samples (n=25) were divided into five groups, negative control (Negative); positive control (Positive); glibenclamide-treated (Glibenclamide); munghurt *Lactobacillus acidophilus*-treated (Munghurt); glibenclamide and munghurt *Lactobacillus acidophilus* combination-treated (Glibenclamide+Munghurt). Diabetes rat was induced by an intraperitoneal injection of alloxan. Munghurt *Lactobacillus acidophilus* was orally administered to normal and diabetic rats for a period of 4 weeks. Blood glucose level was observed on 0 and 28 days. Data were statistically analyzed using Kruskall Wallis and Mann Whitney test and further by one way ANOVA and Tukey post hoc test. **Results:** Munghurt *Lactobacillus acidophilus* decreased blood glucose level significantly on 28 days ($p = 0.001$) with blood glucose level mean of 87.32 ± 5.15 mg dL^{-1} (negative), 233 ± 17.323 mg dL^{-1} (positive), 117.36 ± 8.153 mg dL^{-1} (munghurt), $105.46 \pm 3{,}756$ mg dL^{-1} (glibenclamide) and 105 ± 4.617 mg dL^{-1} (munghurt+glibenclamide) respectively. Munghurt *Lactobacillus acidophilus* combined with glibenclamide had best results at lowering blood glucose in diabetic rats ($p<0.016$). **Conclusion:** Munghurt *Lactobacillus acidophilus* had the effect of the decreasing blood glucose levels and there were a complementary mechanisms between munghurt *Lactobacillus acidophilus* and glibenclamide in lowering blood glucose level in alloxan-induced diabetic rats. However, such effects need to be confirmed on human in clinical condition.

Key words: Blood glucose, diabetic mellitus, *Lactobacillus acidophilus*, mung bean, probiotic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a group of metabolic disease that is characterized by chronic hyperglycemia, insulin deficiency or insulin resistance with classical symptom of polyuria, polyphagia and polydipsia. Diabetes mellitus treatment including insulin and oral anti-diabetics had various side effects especially development of resistance after a certain time period[1,2]. Complementary and alternative medicine in the form of plant-based food commonly used in traditional medicine to treat diabetes nowadays. Mung bean (*Vigna radiate* L.), among all types of seeds, has been recommended as an alternative food for diabetic patients due to its high-fiber content and low glycemic index[3]. Oligosaccharides contained in it can be a source of energy for probiotic bacteria growth. Probiotics are defined as live microorganisms which play an important role in health and disease[4]. Probiotics have decreased blood glucose level through inhibit inflammation and prevented β-cell destruction in animal models[5]. However, human clinical studies using various probiotics have identified a significant lowering glucose effect[6]. *Lactobacillus acidophilus* is the best well-known species of probiotic in Lactic Acid Bacteria (LAB) group. Microorganism metabolic activity results in flavor and aroma production that cause organoleptic properties of fermented foods and inhibits foods harmful bacteria[7-10]. *Lactobacillus acidophilus* play a significant role in production of yoghurt, cheese, fermented cream, milk-based desserts[11]. Exopolysacharides (EPS) play role in microbial cell protection against desiccation, phagocytosis, antibiotics, toxic compounds, predation by protozoans, osmotic stress, solid surfaces adhesion and biofilm formation. Exopolysacharides (EPS) play role in binding to a lectinby cellular recognition[12]. Exopolysacharides (EPS) cause *Lactobacillus acidophilus* is not easy to die and the amount can be maintained. It is expected the combination of mung beans and *Lactobacillus acidophilus* besides being nutritious drinks also potential in lowering blood glucose levels.

Glibenclamide is sulfonylurea class of oral antidiabetic drugs the second generation that has a major effect in increasing insulin secretion from the pancreas[13]. The objectives of this research were to investigate the effect of munghurt *Lactobacillus acidophilus* in decreasing blood glucose levels of diabetic Wistar rat. The objective of this study was to develop a more effective munghurt formula with good taste like yoghurt, in the form of drink, which is more efficient and has an antihyperglicemic effect on diabetic.

MATERIALS AND METHODS

Research was carried out between January and March 2017 at the Animal and Microbiology Laboratories of the Medical Faculty, Universitas Jenderal Ahmad Yani, Cimahi, Indonesia.

Research ethics aspects: Ethical approval was obtained from the Research Ethics Committee Hasan Sadikin Hospital (RSHS) Bandung with No:1165/UN6.C1.3.2/KEPK/PN/2016 and based on the principle of the '3R' AVMA.

Munghurt *Lactobacillus acidophilus* samples: This study used *L. Acidophilus* ATCC 4356 and mung beans varieties from Lembang plantations in West Java, Indonesia according to GACP and were identified in Institut Teknologi Bandung. Tryptone Soya Broth (TSB) medium for rejuvenation of bacteria and De Man Rogosa Sharpe (MSA) medium for bacterial growth medium. Mung beans 500 g soaked with hot water and cold water for eight h. Soaking water replaced every 2 h and mung beans steamed for 15 min. Mung beans were destroyed by the juicer with a ratio of eight parts water and one part of mung beans. Sterilization process continued for 15 min in an autoclave at a temperature 121°C and 2 atm pressure.

Munghurt standards from mung bean (*Vignaradiata*) prepared by Microbiology laboratory, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia. Total plate count of *L. acidophilus* ATCC 4356 10^{14} CFU mL^{-1} stored in the refrigerator at a temperature of 4°C. At the time will be used, munghurt that has been stored in the refrigerator further homogenized using a vortex for 1 min (Fig. 1).

Bacterial re-identification and manufacture of bacterial starter[15]: Bacterial re-identification aimed to determine that bacteria in this experiment were truly of *L. acidophilus* and not contamination. Bacterial re-identification method consists of macroscopic test, microscopic test and biochemistry test. Macroscopic test aimed to identify nature of bacterial colonies. Microscopic test aimed to see Gram staining results, bacterial morphology and formation. Biochemistry tests were used to identify bacteria's ability to produce catalase and to ferment carbohydrates.

Bacterial starter used came from *L. acidophilus* ATCC 4356 bacterial cultures. Bacterial rejuvenation on media Tryptone Soya Broth (TSB) has passed sterilization process. Tube was incubated at 37°C ±0.2 for 24 h after planting bacteria in TSB

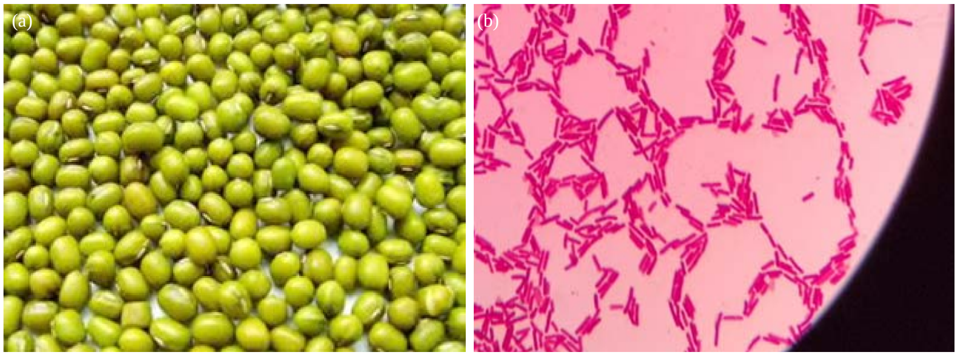

Fig. 1(a-b): (a) Mung bean (*Vignaradiata*) and (b) *L. acidophilus*

with 37-45°C temperature. Gram staining bacteria observation to ensure that starter free from bacteria contaminating. *Lactobacillus acidophilus* cultures taken from TSB on MRS (De Man Rogosa Sharpe) medium, incubated for 48 h and observed with Gram staining. Affirming starter contaminants free by McFarlan 0.250 equivalent to 10^8 in NaCl 0.9%.

Fermentation of mung beans milk: Mungbeans milk 9 mL was added with 1 mL starter suspension. Homogenization process was then performed using a vortex. Fermentation conducted for 40-48 h at a temperature of 37±0.2°C in the incubator. Munghurt stored in the refrigerator at a temperature of 4°C.

Bacterial amount counting: Bacterial amount counting aimed to determine *L. acidophilus* doses given to the treated rats. Total Plate Count (TPC) method used to calculate bacterial amount. Bacterial colonies that eligible to be counted were 25-250 colonies and contaminants free.

Experimental animals: Animal samples of 25 Wistar rats were taken from PT, Biofarma Bandung. The samples (n = 25) were divided into five groups: negative control (Negative); positive control (Positive); glibenclamide-treated from PT. Indofarma (Glibenclamide); munghurt *Lactobacillus acidophilus* treated (Munghurt) and a combination of glibenclamide and munghurt *Lactobacillus acidophilus* treated (Glibenclamide and Munghurt). Diabetes was induced in rats by an intraperitoneal injection of alloxsan monohydrate (*Sawittoku Chemical Laboratories*) 120 mg kg^{-1} intraperitoneally every day for 5 days and the last 3 days were given drinking water containing 10% glucose to produce experimental diabetic condition so that the blood glucose levels >200 mg dL^{-1}. Munghurt *Lactobacillus acidophilus* was orally administered to normal and diabetic rats for a period of 4 weeks.

Diet given to rats and the same dosed each day. Examination of blood glucose levels of Wistar rats performed on 0 and 28 days. Blood comes from the arterial blood vessels behind the eyes (retro-orbital sinus) rats' 0.5 mL blood was collected in micro hematocrit tube and inserted into the centrifuge tube. The blood in the centrifuge tube is rotated for 15-20 min at a speed of 3000 rpm. After that we will get the blood plasma to be used for checking blood glucose levels using a spectrophotometer with a wavelength 500 nm. Blood glucose levels were measured by the method of GOD-PAP[16].

Statistical analysis: Data were statistically analyzed by paired t-test for the analysis of wistar rats' blood glucose levels before and after alloxan induced and one way ANOVA and Tukey post hoc test for effect of munghurt to blood glucose levels, after affirming the normality and homogeneity of variances assumptions of data sets. p values of <0.05 were considered to be significantly different[17].

RESULTS

Bacterial re-identification: Macroscopic examination results on MRS medium were white colony, pinpoint, colony diameter 0.5-1.5 mm, convex elevation, smooth-edged and grayish-white color. Bacterial colonies aroma result was sour aroma. This was in accordance with characteristics Lactobacillus colony morphology parameter. Microscopic examination results were Gram positive rod and chain formation. The examination results in accordance with microscopic characteristic of *Lactobacillus acidophilus* (Fig. 2).

Biochemistry examination for catalase test showed negative results. Catalase test aimed to identify bacteria's ability to transform H_2O_2 into H_2O and O_2 using the enzyme catalase. The results obtained in catalase test were negative, characterized by no oxygen bubbles formation because

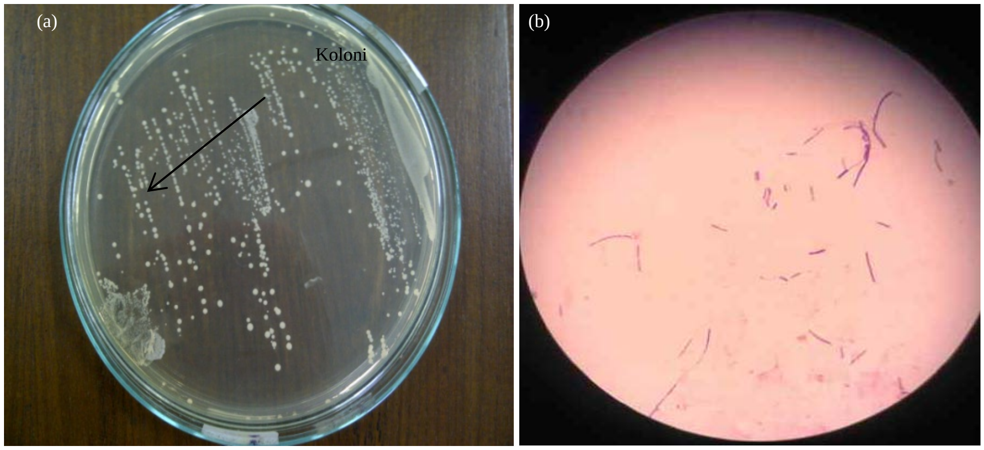

Fig. 2(a-b): Macroscopic and microscopic examination results of *L. acidophilus* (a) Colony morphology and (b) Gram staining results

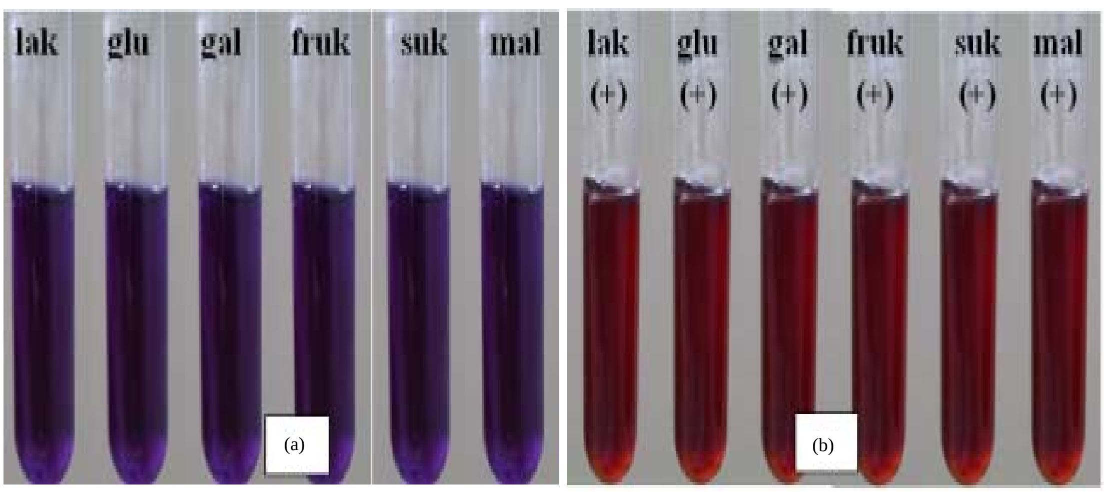

Fig. 3(a-b): Carbohydrates fermentation by *L. acidophilus* (a) Before fermentation and (b) After fermentation

of *Lactobacillus acidophilus*. Biochemistry test for carbohydrate fermentation was positive. Brom Cresol Purple (BCP) as an indicator showed positive results forlactose, glucose, galactose, fructose, sucrose and maltose marked with purple changes to red color. It was caused by bacteria which produced lactic acid which can lower pH and became acidic. It can be concluded that all of the results suitable for *L. acidophilus* without contamination (Fig. 3).

***Lactobacillus acidophilus* dose:** Colony calculation results were average number of colonies with three times repetitions. These results were multiplied by dilution factor and expressed in Colony Forming Units per mL (CFU mL^{-1}). Based on colony calculation results *L. acidophilus* dose was 6.33×10^{17} CFU mL^{-1}.

Effect of munghurt *Lactobacillus acidophilus*to blood glucose levels: Effect of munghurt *Lactobacillus acidophilus* on blood glucose levels were seen using one way ANOVA test on the results of blood glucose levels on 0 day and 28 days. Blood glucose level was not found to be decreased, both in the positive control and the negative control groups. The different results obtained in glibenclamide group, munghurt group and glibenclamide and munghurt group which showed a decrease in blood glucose levels after treatment (Fig. 4).

To determine whether a significant decrease in its blood glucose levels, performed statistical analysis dependent t-test analysis. The treatment group which was given munghurt *Lactobacillus acidophilus* showed a decrease in blood glucose levels were significant before and after treatment with an average difference of 117.36 mg dL^{-1} ($p<0.05$) (Table 1).

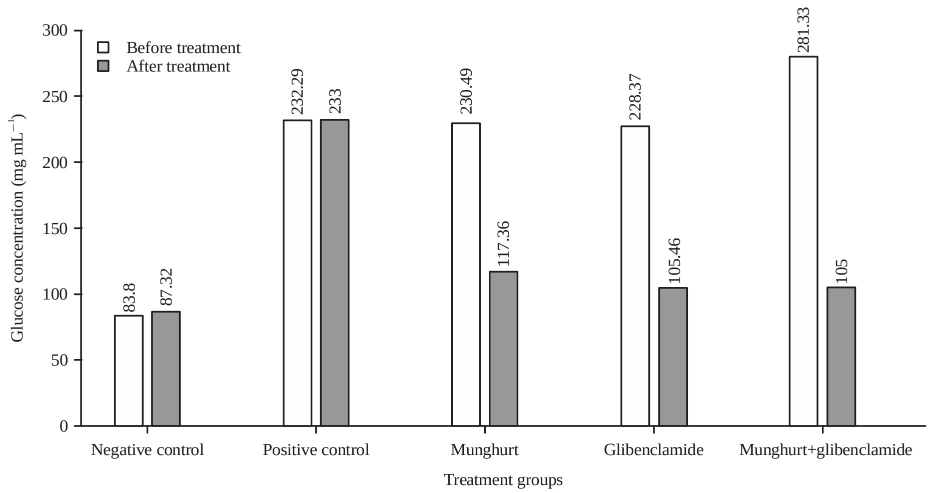

Fig. 4: Effect of munghurt in blood glucose levels before and after treatments compared with other treatment groups

Table 1: Effect of munghurt in lowering blood glucose compared with other treatment groups

Treatment groups	Blood glucose levels before treatment (mg dL^{-1})$^{\#}$	Blood glucose levels after treatment (mg dL^{-1})$^{\#}$	Difference (mg dL^{-1})	p-values
Negative control	83.80±5.813	87.32±5.615	-3.523	0.007
Positive control	232.29±17.373	233.00±17.323	-1.246	0.027
Group 1	230.49±27.288	117.36±8.153	113.134	0.001*
Group 2	228.37±14.125	105.46±3.756	122.919	0.000*
Group 3	281.33±27.637	105.00±4.617	176.280	0.000*

Paired t-test, *p<0.05 shows values are statistically significant, #Values are given as Mean±SD

Table 2: Differences on blood glucose levels between negative control, positive control, munghurt and glibenclamide group

Groups	Comparison groups	p-values
Negative control	Positive control	0.047*
	Munghurt	0.009*
	Glibenclamide	0.009*
	Munghurt+glibenclamide	0.009*
Positive control	Negative control	0.047*
	Munghurt	0.009*
	Glibenclamide	0.009*
	Munghurt+glibenclamide	0.009*
Munghurt	Glibenclamide	0.347
	Munghurt+glibenclamide	0.016*
Glibenclamide	Munghurt+glibenclamide	0.016*

Post hoc Mann Whitney test, *p<0.05 shows values are statistically significant

Effect of munghurt *Lactobacillus acidophilus* compared with glibenclamide to blood glucose levels: Effect of munghurt *Lactobacillus acidophilus* compared with glibenclamide to the blood glucose levels was done by comparing the blood glucose levels of each group with glibenclamide result on 28 days (Table 2).

DISCUSSION

Munghurt *Lactobacillus acidophilus* have showed effective decrease in blood glucose levels in diabetic rats. Munghurt *Lactobacillus acidophilus* can reduce diabetic rats blood glucose level associated with E-cadherin/β-cetaninin probioticsstimulate immune system by improve intestinal permeability and increase intestinal normal flora such as gram-positive bacteria. It will inhibit the growth of Gram-negative bacteria and decrease the amount of lipopolysaccharide derived from Gram-negative bacteria. It will reduce systemic inflammatory reaction including damaging the beta cells of the pancreas that produce insulin[18]. Besides that, substance content of flavonoids in mung beans have the effect of lowering oxidative stress was caused by alloxan induced by binding with free radicals as well as giving the effect of inhibiting GLUT 2 in intestinal epithelial cells, so that the absorption of glucose by the intestines become blocked[19].

In this study it is shown that alloxan can cause damage to the pancreatic β cells which increase blood glucose levels in diabetic rats. This was consistent with Rachmat *et al.*[20] that the administration alloxan of 120 mg kg^{-1} b.wt., intraperitoneal increased blood glucose levels cause the metabolic oxidation-reduction which produces free radicals and radical alloxan. These free radicals and radical alloxan cause damage to the pancreatic β cells. The islets of langerhans in the pancreas showed reduction in mass of cells, starting to shrink, crushed, even disappeared. As a result of the pancreatic β cell damage, then the pancreas was unable to produce the hormone insulin that causes a state of hyperglycemia in the body. Hyperglycemia also aggravate from β cell damage[21]. Hyperglycemia condition tends to increase the formation of free radicals. Reactive Oxygen Species (ROS) were form through m etabolic pathways auto-oxidation glucose such as glucose, metilglicosal formation metabolism and oxidative phosphorylation. Excessive reactive oxygen species can cause damage to pancreatic β cells[22].

Results of comparison between glibenclamide group and combination munghurt *Lactobacillus acidophilus* and glibenclamide group, obtained significant results with p = 0.016 ($p<0.05$). This indicates that administration of combination glibenclamide and munghurt *Lactobacillus acidophilus* better in lowering blood glucose levels in diabetic rats when compared to glibenclamide group. A decrease in blood glucose was likely caused by presence of a cooperative mechanism between glibenclamide and munghurt *Lactobacillus acidophilus*. Glibenclamide stimulates pancreatic β cells to increase insulin secretion. Glibenclamide able to stimulate insulin secretion stored in pancreatic β cells granules by interacting with ATP-sensitive K channels in pancreatic beta cells membrane. It was assisted by *L. acidophillus* on munghurt which have the effect of lowering blood glucose levels by improving intestinal permeability and suppress inflammation by inhibiting pro-inflammatory cytokines in order to minimize pancreas beta cells damage. Flavonoids contained in green beans on munghurt *Lactobacillus acidophilus* have the effect of lowering oxidative stress caused by alloxan induced by binding with free radicals. Flavonoids contained in green beans on munghurt *Lactobacillus acidophilus* can inhibit GLUT 2 in intestinal epithelial cells which inhibit glucose absorption[17]. Mung beans have a low glycemic index value: 28.87 (<55)[23]. Foods consumptions with low glycemic index value can decrease blood glucose levels slowly so that helps control blood glucose levels[24]. Synergies mechanism between glibenclamide and munghurt *Lactobacillus acidophilus* result in decreasing in blood glucose rats meaningful and better when compared with glibenclamide or munghurt *Lactobacillus acidophilus* individually.

Effect of munghurt *Lactobacillus acidophilus* on glucose levels ratio in 28 days of treatment showed a very significant change. The results showed that munghurt can improve diabetic rat condition, causing blood glucose levels return to near normal conditions.

CONCLUSION AND FUTURE RECOMMENDATION

This study showed that munghurt *Lactobacillus acidophilus* had decreased diabetic rats' blood glucose levels. There are complementary mechanisms between munghurt *L. acidophilus* and glibenclamide in decreasing diabetic rats' blood glucose level. It is stated that munghurt *Lactobacillus acidophilus* showed effect on blood glucose levels decrease in Wistar rats induced alloxan on 28 days. Hence it may be a useful antihyper glycemic in cases of diabetes mellitus and can be used as an adjuvant along with clinically effective antidiabetic agent. Further research is needed to determine the role of munghurt *Lactobacillus acidophilus* in lowering blood glucose levels in pancreas and hepar histopathology change.

SIGNIFICANCE STATEMENTS

This study discovered the possible effect of mung bean (*Vigna radiata* L.) that can be beneficial for antihyperglicemia drugs. This study will help the researcher to uncover the critical areas of an alternative food for diabetic patients due to its high-fiber content and low glycemic indexmung bean (*Vigna radiata* L.) munghurt *Lactobacillus acidophilus* that many researchers were not able to explore. Thus a new theory on complementary mechanisms between munghurt *L. acidophilus* and glibenclamide in lowering blood glucose level in diabetes mellitus patient may be potential in health treatment with natural products.

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