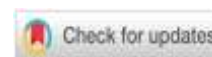




Moringa oleifera gum effect on blood sugar levels and rat pancreas histology induced by Streptozotocin



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ABSTRACT

Moringa gum is a product that people can use as an anti-hyperglycemic agent. This study aimed to determine the effectiveness of Moringa gum in reducing blood sugar levels and improving the histology structure of the rat pancreas induced by streptozotocin (STZ). This study used 21 male rats divided into 3 groups including control, diabetes (STZ), and diabetes (STZ + 3% Moringa gum). STZ treatment at a single dose (45 mg/kg BW) was given intraperitoneally, while Moringa gum was given through drinking water for 2 weeks. Blood sugar levels were measured on days 0, 7th, 14th, 21st, and 28th. The results showed that STZ administration induced diabetes in rats with blood sugar levels of 487 mg/dL on the 14th day. Moringa gum administration was able to reduce blood sugar levels on days 21st and 28th respectively to 306 and 234 mg/dL. The histology structure of the pancreas of rats treated with gum also showed improvement. This study concludes that Moringa gum is effective in lowering blood sugar levels in rats even though it still does not show normal blood sugar levels, and can repair STZ-induced damage to the histology structure of the rat pancreas.

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INTRODUCTION

Glucose is a simple carbohydrate that can be absorbed in the bloodstream. Blood sugar levels are closely related to diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by increased blood sugar levels above normal (hyperglycemia) due to impaired regulation of insulin levels in the body (Hidayaturrohman et al., 2020). Diabetes mellitus can be caused by two factors, the first is insulin deficiency so that the body is unable to control blood sugar and the second is insulin resistance, the condition of the body's cells being insensitive to insulin secreted by the pancreas (Mc Clung et al., 2004).

As a research model, rats were induced by *streptozotocin* (STZ). STZ is used to induce diabetes in experimental animals because they can maintain the diabetic condition (Eleazu *et al.*,



2013). STZ has a half-glucose structure that can bind into *glucose transporter 2* (GLUT2) so that it can enter the β pancreas cell and cause a hyperglycemic response (Szkudelski, 2001). STZ causes an increase in *reactive oxygen species* (ROS) which can result in increased oxidative stress, especially in pancreatic β cells. Pancreatic β cells experience degeneration so that insulin secretion decreases increasing blood sugar levels because the body's cells cannot absorb glucose optimally (Lai, 2018; Hamdan et al., 2011; Husni et al., 2016). Blood sugar levels in diabetes sufferers can be controlled by taking medication that is generally consumed continuously (Trevor et al., 2009). However, in the long term, sufferers can experience several side effects, which has prompted a lot of research to be carried out regarding the use of natural ingredients to regulate blood sugar levels in diabetes sufferers (Wang et al., 2013).

One of plant that can be used as an alternative to stabilize blood sugar levels in diabetes mellitus sufferers is the Moringa plant (*Moringa oleifera*). Many studies have been carried out regarding the efficacy of leaf, stem, fruit, and seed extracts of *M. oleifera* as a treatment ingredient for diabetes mellitus sufferers (Vargas-Sanches et al, 2019). However, but its potential has not been studied for the gum part. Research on the effect of gum on rat blood sugar levels has been carried out on Arabic gum by Nagar (2017) and Wiyono et al. (2021), but has never been studied on *Moringa* gum.

In general, *gum* contains the main chemical compounds such as *D-galactose*, *L-arabinose*, *L-rhamnose*, *D-glucuronic*, and *4-O-methyl-D-glucmic acid* (Mohammad, 2015). With this content, the gum is included in high-fiber foods which can play a role in inhibiting glucose absorption in the small intestine, so it is hoped that it can reduce hyperinsulinemia (Glover et al., 2009; Nasir et al., 2014). Apart from polysaccharides, there are also several secondary metabolite compounds in gum. During this time the use of *Moringa* gum. It is only known as a source of nutrition which is also used as a traditional medicine, so the potential of *M. oleifera* gum still needs to be studied specifically as lowering blood sugar levels in diabetes mellitus sufferers.

RESEARCH METHODS

Research Design

This research used a Completely Randomized Design (CRD). The test animals used were 21 male rat (*Rattus norvegicus* L)Wistar strain. Induction of diabetes is carried out by injecting a single dose of STZ (45 mg/kg body weight) and then the blood sugar level is measured when the blood sugar level has reached ≥ 165 mg/dL followed by the administration of 3% *Moringa* gum. Mice were divided into 3 groups and 7 replications, with the following doses:

Group 1: Rats were not induced STZ and without *Moringa* gum

Group 2: Rats were induced STZ without *Moringa* gum.

Group 3: Induced mice STZ and given a gum *Moringa* gum.

Observations were made on blood sugar levels and pancreatic histology. Blood sugar level was analyzed statistically using T-test and analysis of variance, further conducted using DMRT. The pancreas histology was analyzed descriptively.

Population and Samples

This research used male Wistar strain rats aged 8-10 weeks with a body weight of 150-200 grams 8 weeks in healthy condition. Rat was kept in cages with feed and drink was given ad libitum. Rat were grouped according to the treatment and repetition carried out in Completely Randomized Design.

Instruments



The research instrument includes cages, syringes, glucocheck and strips, surgical tools, dissecting boards, ovens, rotary evaporators, automatic stainers, microscopes, and optilabs.

Procedures

This research was begun by rat acclimatization, STZ dan Moringa gum treatment, blood sugar level measurements, pancreas histology preparations, observation and data analysis. Blood sugar levels are measured on 0, 7th, 14th, 21st, and 28th day. The research procedures include all the steps carried out during the research described in Figure 1.

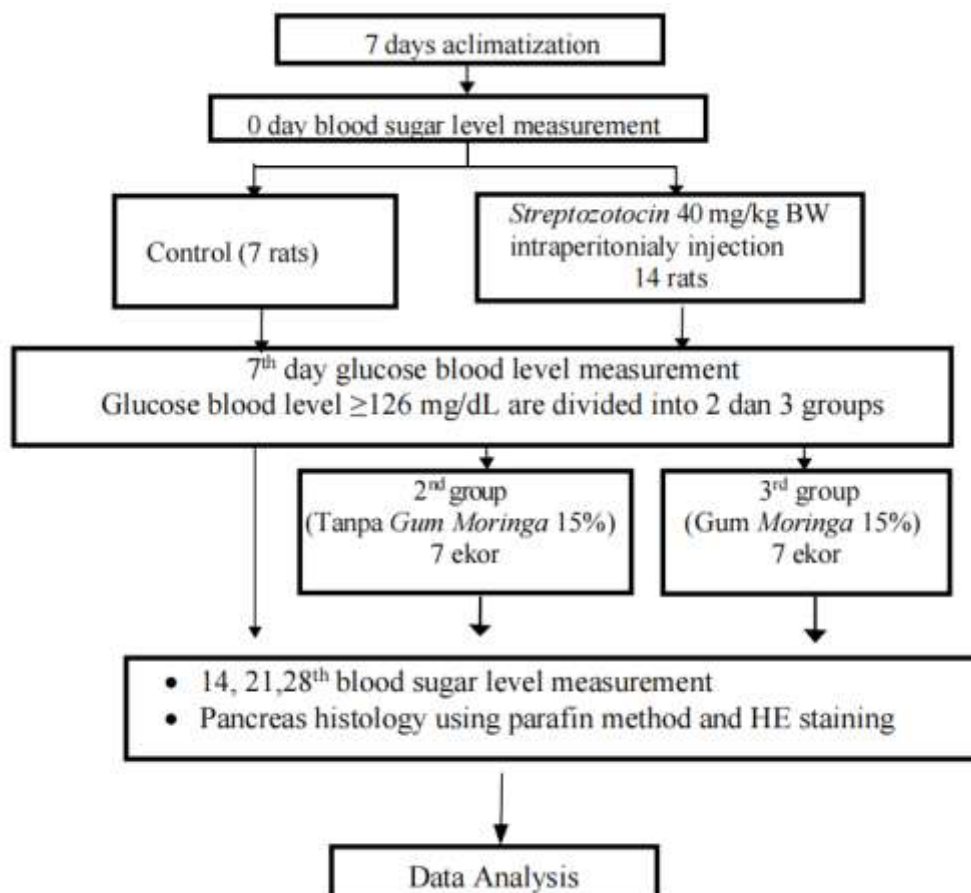


Figure 1. The flow diagram of research implementation

Data Analysis

Observation data were analyzed statistically using T-test to know the effect of STZ injection on blood sugar levels. Afterward continued by analysis of variance (F test) to know the effect of Moringa gum treatment and further tests were conducted using Duncan's multiple range test (DMRT).

RESULTS

In this study, STZ induction was carried out to increase rat blood sugar levels constantly and become diabetic. Blood sugar levels were measured on day 0 and then blood sugar levels were measured again on day 7th after STZ induction. Data on the results of measuring blood sugar levels can be seen in Table 1. The results of measuring the mean \pm SD of blood sugar levels on days 14th, 21st, and 28th can be seen in Table 2. The results of observations of the histological structure of the rat pancreas can be seen in Figure 1.

Table 1. Average blood sugar levels of mice on day 0 and day 7th after STZ administration

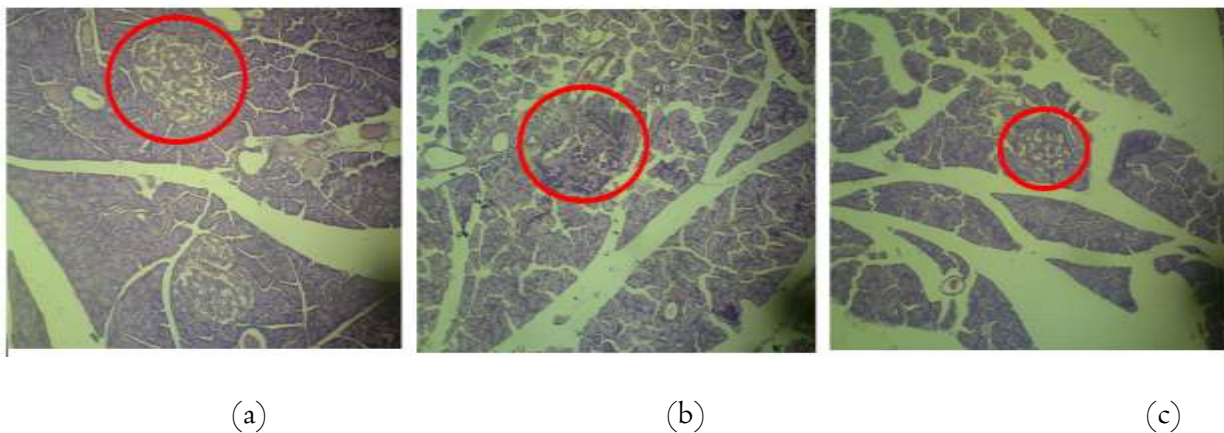
Treatment	Blood sugar level ($\bar{x} \pm SD$) (mg/dL)	
	Day 0	Day 7 th
Control	105 \pm 15,59 ^a	111 \pm 14,11 ^a
STZ	108 \pm 13,17 ^a	437 \pm 46,22 ^b

Numbers followed by different letters in the same column are significantly different based on the DMRT test at the level ($p < 0.05$).

Table 2. Mean \pm SD blood sugar levels of hyperglycemic mice after administration of gum *Moringa* 3% for 2 weeks on days 14th, 21st, and 28th

Group	Blood sugar level (mg/dL) ($\bar{x} \pm SD$)			
	Day 7 th	Day 14 th	Day 21 st	Day 28 th
Control	111 \pm 14 ^a	125 \pm 4,47 ^a	128,42 \pm 7,45 ^a	114 \pm 8,40 ^a
STZ	437 \pm 46,22 ^b	487 \pm 66,01 ^b	479,42 \pm 46,31 ^b	420,28 \pm 63,24 ^b
STZ + Gum <i>Moringa</i> 3%	364 \pm 19,23 ^b	479,14 \pm 66,29 ^b	306,42 \pm 39,47 ^a	234,14 \pm 29,19 ^a

Numbers followed by different letters indicate significant differences based on the DMRT test at the level ($p < 0.05$).



a. Pancreas histology in control group

b. Pancreas histology in STZ-treated group

c. Pancreas histology in STZ followed by 3% *Moringa Gum* treatment group

The red circles showed the structure of the Langerhans islands

Figure 2. Histology of the rat pancreas (10x10 magnificant)

DISCUSSION

Table I showed that the average blood sugar levels before STZ administration (day 0) in the control group and the STZ group were not significantly different, namely within the normal range: 113 \pm 12 mg/dL and 107 \pm 10 mg/dL respectively. Normal blood sugar levels in rats range between 50-135 mg/dL (Hidayaturohmah et al., 2020). Meanwhile, hyperglycemic blood sugar levels in mice are shown to the blood sugar levels of more than 200 mg/dL (Vasukeshetti et al.,

2016). Measurement of blood sugar levels on day 7th in the control group showed levels of 111 ± 14 mg/dL, which is still within the range of normal blood sugar levels, and the group of rats injected with STZ showed increasing in blood sugar levels more than 387 ± 37 mg/dL so that classified into the diabetes group.

In this case, it was shown that STZ induction was able to increase blood sugar levels in rats which indicated diabetes. STZ increased *nitric oxide* (NO) synthesis resulting in the degeneration of β cells pancreas (Eleazu *et al.*, 2013). *Nitric oxide* (NO) is included in *reactive oxygen species* (ROS) which can increase oxidative stress in β cells pancreas so it can reduce the ability of the pancreas to secrete insulin (Lai, 2018; Hamdan *et al.*, 2011). Decreased insulin secretion causes blood sugar levels in the body to increase, thought to be caused by pancreatic β cells undergoing apoptosis (Husni *et al.*, 2016). From these results, the treatment was continued by gum administration at a dose of 3% for two weeks.

The results of measuring the mean \pm SD of blood sugar levels on days 14th, 21st, and 28th after gum administration can be seen in Table 2. On the 14th day, the blood sugar level of mice with STZ treatment and STZ mice given gum *Moringa* was 3% increased compared to day 7th. Blood sugar levels in the two groups did not show any significant differences. This is caused by *Moringa* gum first given on the 14th day. On the 21st day, there was a decrease in blood sugar levels in the diabetic rats that were given 3% *Moringa* gum, while diabetic mice that were not given 3% *Moringa* gum showed blood sugar levels that remained high, despite a slight decrease. There is a significant difference between the two groups. Likewise, on the 28th day, there was a decrease in blood sugar levels in both groups of diabetic rats treated with STZ and STZ with gum. However, the decrease in blood sugar levels in rats treated with gum was more than in rats treated only with STZ. This showed that 3% *Moringa* gum can act as an antihyperglycemic compound even though the sugar levels of diabetic rats are still high (the mice are still diabetic).

Decreased blood sugar levels in the group of mice given *Moringa* gum were thought to be due to *Moringa* gum being an *undigestable polysaccharide*. *Moringa* gum contains salts, and polysaccharides including L-arabinose, D-galactose, L-Rhamnose, and D-Glucuronic acid. These polysaccharides have long chains and are highly branched with various types of branching. Colon bacteria ferment *Moringa* gum slowly until it produces *short-chain fatty acid* (SCFA) (Srinivasan *et al.*, 2005). The results of fermentation in the form of SCFA are divided into *acetate* (C2), *propionate* (C3), and *butyrate* (C4) (Cook & Selin, 1998). *Butyrate* stimulates increased secretion of *glucagon-like peptide-1* (GLP-1) by L cells so that GLUT 4 expression increases (Besten *et al.*, 2013; Penacarrillo *et al.*, 2001). GLUT 4 acts as a glucose transporter so that it can enter skeletal muscle cells. GLUT 4 expression increases due to GLP-1 activation *phosphoinositide3-kinase* (PI3K) in skeletal muscle cells so that glucose entering the skeletal muscle cells also increases (Green *et al.*, 2012)] Thus, blood sugar levels decrease. The decrease in blood sugar levels in mice treated with Gum still did not show normal blood levels. This is because the gum dose given was not high enough (only 3%). Previous research results showed that giving gum Arabic at a dose of 15% was able to reduce the sugar levels of STZ-induced diabetic rats (Wiyono *et al.*, 2021).

The results of observations of the histology structure of the rat pancreas can be seen in Figure 2. The histology structure of the pancreas of control mice shows that the islets of Langerhans have a large size and a compact cell structure. Meanwhile, in rats treated with STZ, the pancreatic islets of Langerhans appeared to have smaller structures and damaged structures. The damage experienced is thought to be due to the influence of STZ administration which causes necrosis on β pancreas cell. *Moringa* gum administration appears to be able to regenerate this damage. It can be seen in Figure 2 that the pancreatic islets of Langerhans in mice treated with gum *Moringa* The 3% appears more compact, although its size does not yet resemble the control. The results of observations of the histology structure of the pancreas showed that in the pancreas

of rats treated with STZ, there was damage to the islets of Langerhans, which was characterized by necrosis of the islets of Langerhans cells and the introduction of pancreatic acinar between the cells of the islets of Langerhans. Research shows that STZ is cytotoxic through its indirect role as a Nitric Oxide (NO) donor which can cause DNA damage in pancreatic β -cells in mice. NO molecules are formed when STZ undergoes metabolism in cells. Apart from NO, STZ can also cause the formation of ROS which can also cause DNA fragmentation and other cell damage (El Nagar, 2017).

Compared with controls, the cells of the islets of Langerhans appeared to be arranged compactly and regularly and there were no cells experiencing necrosis. In 3% *Moringa gum* treatment group showed that the islets of Langerhans structure improved so that the structure was more compact than in the STZ treatment (Figure 2). In this case, it is allegedly because *Moringa gum* has antioxidant activity that can react with free radical compounds (ROS) so that it can reduce damage to the structure of the pancreatic islets of Langerhans in diabetic mice. This is by research which states that *Moringa gum* contains phytochemical compounds including alkaloids, saponins, and oils (Annaamalai *et al.*, 2017). Saponin acts as an antioxidant by reducing superoxide through the formation of hydroperoxide so that it can prevent lipid damage (lipid peroxidation) (Hasan *et al.*, 2022). Meanwhile, alkaloids can act as antioxidants by donating H atoms to stabilize free radicals (Widiastuti *et al.*, 2021). In this way, it can prevent lipid peroxidation which can cause damage to the β cells of the islets of Langerhans in the rat pancreas.

CONCLUSION

The conclusion of this research is, that giving gum *Moringa* was able to reduce blood sugar levels in diabetic mice due to STZ induction, however, this reduction in sugar levels did not indicate normal blood sugar levels. Giving *Moringa gum* also showed an improved effect on the histology structure of the pancreas.

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