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The hypoglycemic and regenerative effect of the pancreas using instant porridge mix of pumpkin and brown rice flour on diabetic rats

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ABSTRACT

Diabetes is a congenital disease resulting from inefficiencies in insulin production and activities. Instant porridge mixed with pumpkin and brown rice (instant porridge mix) can be a functional food to lower blood sugar. This study aimed to determine the hypoglycemic activity and the ability of instant porridge mix to regenerate pancreatic beta cells in diabetic rats. Diabetes was induced by Streptozotocin (STZ). Instant porridge mix was used to substitute the standard feed AIN-93 at 0, 10, 20, and 30% levels. The hypoglycemic activity test used 30 Sprague Dawley rats assigned to five groups with six each. The groups were (1) normal rats fed with standard feed AIN-93, (2) DM/diabetes mellitus rats fed with AIN 93 feed, (3) DM rats fed with 10% instant porridge mix, (4) DM rats fed with 20% instant porridge mix, and (5) DM rats fed with 30% instant porridge mix. The treatment was carried out for twenty-eight days, and blood sampling was carried out at seven-day intervals for blood analysis to determine glucose levels. At the end of the study, the levels of MDA (malondialdehyde) and blood glucose in the liver of the rats were also analyzed. A histopathology test was also done on the pancreas. The results showed that feed substitution (20%) with instant porridge mix significantly ($p < 0.05$) reduced the level of blood glucose from 271.81 to 99.66 mg.dL⁻¹ in DM rats. In conclusion, DM rats fed with 20% instant porridge mix were the best treatment for hypoglycemic and regenerative effects of the pancreas.

Keywords: instant porridge mix, pumpkin, brown rice, pancreatic β cells, hypoglycemic, diabetic rats.

INTRODUCTION

Diabetes mellitus (DM) is a chronic degenerative disease resulting from low insulin production by the pancreas or poor insulin performance. DM is characterized by chronic high blood sugar (hyperglycemia) due to impaired insulin production, secretion, or insulin resistance. The increasing number of people suffering from DM and the accompanying complications have made DM a very concerning disease. In 2014, 12 million people in Indonesia suffered from DM, reportedly [1] the third most deadly disease after stroke and heart disease. Another study defines DM as a chronic, multi-factorial metabolic disorder generally characterized by disorders of carbohydrate, protein, and lipid metabolism resulting from damage to the action of insulin, secretion of insulin, or both [2]. The prevalence of world diabetes in 2004 was 2.8% and is estimated to reach 4.4% by 2030 [3]. In 2014, WHO reported a 9% diabetes prevalence among the human population, and therefore, diabetes must be treated immediately. Various kinds of antidiabetic medicine have been used to treat diabetes, including oral antidiabetic drug, which reportedly causes side effects such as liver problems, diarrhoea, and lactic acidosis [4]. Today, there has been considerable research on the potential natural ingredients for safer treatments of diabetes. Vegetable bearing hypoglycemic potentials such as pumpkin (*Cucurbita pepo* L) has been made into flour and fed to rats to reduce blood sugar levels [5]. Pumpkin contains bioactive compounds such as phenolic, proteins, peptides, sterols, terpenes, and polysaccharides [6] and hypoglycemic properties and antidiabetic effects [7]. Ju and Chang [8] found that pumpkin flour as feed successfully increased plasma insulin and reduced glucose levels

significantly. Various pumpkins contain pharmacological characteristics, including antioxidant, hepatoprotective, and lipid-lowering abilities [9], antidiabetic abilities [10], antimicrobial and anti-carcinogenic [11]. The mechanism of blood sugar depletion is through the accelerated release of glucose from circulation, which is closely related to the work of the heart, and by accelerating the filtration and excretion of the kidneys. By this, urine production increases, and the rate of glucose excretion through the kidneys increases, thereby decreasing glucose levels in the blood. Rice is food and a source of energy, and the source of protein, vitamins, and minerals that are beneficial for human health. There are several types of rice in Indonesia based on colors, such as white rice, black rice, glutinous rice, and brown rice. Brown rice is generally sold without brushing but ground into cracked rice with the husk still attached to the endosperm. While brown rice bran is rich in natural oils, essential fats, and fiber [12], organic brown rice contains 6.5% moisture, 1.8% fat, and 73.8% starch [13].

Pumpkin and brown rice instant porridge (instant porridge mix) is the potential functional food to lower blood sugar in people with diabetes. This study was conducted to determine the effect of different levels of pumpkin and brown rice instant porridge on antidiabetic activity and the capacity of the porridge mix to regenerate β -pancreatic cells in rats.

Scientific Hypothesis

This study hypothesizes that pumpkin and brown rice instant porridge could serve as a novel functional food to control DM by depleting blood glucose and generating β -pancreatic cells in diabetic rats.

MATERIAL AND METHODOLOGY

Chemical substances

All chemical substances used in this study were analytical grade. The Streptozotocin (STZ) was purchased from Sigma-Aldrich (Germany), and the reagents included GOD-PAP (Glucose Oksidase-Phenol Amino peroxidase) and Nicotinamide (Na). The insulin kit (Rat) Elisa was manufactured by DRG Catalog No EIA 2048. We used AIN-93 standard feed, which consisted of casein, fiber, soybean, choline bitrate, AIN 93 MX, corn starch, L-cystine, and AIN 93VX.

Description of the Experiment

Sample preparation: The pumpkin was peeled, seeded, and cut to a size of 2 x 2 x 2 cm. Then, the pumpkin was mixed with brown rice (75:25 w/w) in a blender, added with 50 mL of distilled water, and blended. The mixture was oven-dried at 160 °C for 15 minutes to make instant porridge [14] that would substitute the feed for the Sprague Dawley rats.

Preparation of feed formulation: The feed formulation used is present in Table 1.

Table 1 Feed formulations for experimental rats.

Composition	Feed treatment (g)			
	Standard and 0%	10% Instant porridge	20% Instant porridge	30% Instant porridge
Maizena	620.69	520.69	420.69	320.69
Instant porridge*	0	100	200	300
Casein	140	140	140	140
Sucrose	100	100	100	100
Oil	40	40	40	40
Fiber	50	50	50	50
AIN 93 MX	35	35	35	35
AIN 93 VX	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5

Note: DM – Diabetes mellitus.

Animal experiment

We used 30 male Sprague Dawley rats aged two months and weighed 230 grams to be reared in stainless steel cages. Before the experiment, the rats were adapted to standard feed AIN-93 for three days, and drinking water was provided ad libitum. AIN-93 was the standard feed used in this study [15]. The rats were reared in a room with natural lighting at room temperature. After the adaptation period, the rats were fed with high cholesterol feed

for seven days by substituting the maizena with 20 g of cholesterol and 2 g of cholic acid. After seven days, 24 Sprague Dawley rats were induced with diabetes intravenously using Streptozotocin/STZ dissolved in 3 mL citrate buffer administered at a dose of 65 mg.kg⁻¹ body weight of the rat [16]. Five days after the administration, blood samples were drawn from the rats for day 0. All 30 rats were rationed to 5 treatment groups, namely (1) normal rats fed with standard feed AIN-93, (2) 0% DM rats fed with AIN 93 feed, (3) DM rats fed with 10% pumpkin and brown rice instant porridge mix (4) DM rats fed with 20% instant porridge mix, and (5) DM rats fed with 30% instant porridge mix. Food and water were provided ad libitum, the treatment was carried out for twenty-eight days, and blood samples were collected at seven days (days 0, 7, 14, 21, and 28) for blood glucose analysis using Insulin Elisa Kit (U-Cloud-Clone Corp). Before abdominal surgery, the rats were anaesthetized using diethyl ether to obtain the liver and pancreas. The liver was subjected to analysis using the thiobarbituric acid reactive substance (Cayman, USA) to determine MDA levels.

Histological and immunohistochemical analysis (IHC)

The pancreas was cleaned and fixed in 10% formalin solution and dehydrated and dipped in a paraffin solution before slicing to 7 μm thick using a microtome. The incised portion was stained with hematoxylin-eosin, and the Island of Langerhans found on the stained pancreas was subjected to IHC analysis using the Histofine Mouse Stain Kit. Langerhans' dark brown islets indicate insulin presence, whose intensity was analyzed semiquantitatively.

Statistical Analysis

The statistical analysis was performed using SPSS version 24 (SPSS Inc., Chicago, Illinois, USA), and significant differences were tested using the Duncan Multiple Range Test (DMRT) at the 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Weight of rats and feed intake

The average body weight of the rats at the start of the experiment was 185.99 ± 7.98 grams (Figure 1), then increased to 209.50 ± 7.21 g after the adaptation period. However, after diabetes treatment, the body weight of control diabetic rats (DM rats fed on standard feed AIN-93 or 0% instant porridge mix) decreased from day 0 to day 28. Thus, the control diabetic rats experienced the most weight loss throughout the experiment. Meanwhile, increased body weight was observed among normal rats with standard feed and not significantly different ($p < 0.05$) body weight among DM rats fed with 10, 20, and 30% instant porridge mix on the 28th day. This study revealed that pumpkin and brown rice instant porridge mix could improve metabolism in the rat compared to DM rats fed on standard feed or 0% instant porridge mix.

The average feed intake of the rats during 28 days showed that feed intake was not significantly different ($p < 0.05$) between normal rats (11.78 ± 0.36 g) and DM rats fed on instant porridge mix at 0% (13.7 ± 0.19 g), 10% (11.21 ± 0.25 g), 20% (11.36 ± 0.31g) and 30% (11.70 ± 0.59 g). The highest feed intake was in DM rats fed with 0% instant porridge mix. Therefore, in diabetic rats, standard feed (0% instant porridge mix) could not improve body weight and diabetes conditions because the rats' body metabolism was affected, which increased feed demand but lowered weight gain.

Blood glucose levels of rats

The blood glucose levels are shown in Table 2 and Figure 2. The initial blood glucose level ranged from 87.26 to 88.96 mg.dL⁻¹, suggesting an average serum glucose level before the induction of diabetes with STZ. According to Nichols [17], blood glucose level for normal rats ranges between 62 and 175 mg.dL⁻¹. After the adaptation period, DM rats fed on 0, 10, 20, and 30% instant porridge mix were induced with diabetes. Three days after the induction, the blood glucose level increased significantly ($p < 0.05$) from 265.48 to 276.84 mg.dL⁻¹, whereas in the normal rats, significant differences ($p < 0.05$) were not observed. Treatment with STZ resulted in diabetic conditions in the rats and could damage the β-pancreatic cells. The glucose transporter (GLUT2) enables the transfer of STZ into β-pancreatic, rendering necrosis in the insulin-secreting cells [16].

The effects of pumpkin and brown rice instant porridge mix on the level of blood glucose in DM rats are shown in Figure 2. During the 28-day experiment, the glucose levels of the blood serum decreased across treatment except for the control DM rats (rats fed with 0% instant porridge mix). Treatment with pumpkin and brown rice instant porridge mix generally reduced the blood glucose levels in which the higher percentage of treatment, the lower the glucose level (Table 2). In other words, the intake of pumpkin and brown rice instant porridge can improve diabetic conditions, and the antidiabetic capacity increases with the intake level. Aukanit and

Sirichoworrakit [18] reported that feeding rats with 2 g.kg⁻¹ pumpkin flour could better reduce glucose levels (from 104.2 to 98.75 mg.dL⁻¹) than 1 g.kg⁻¹ pumpkin flour.

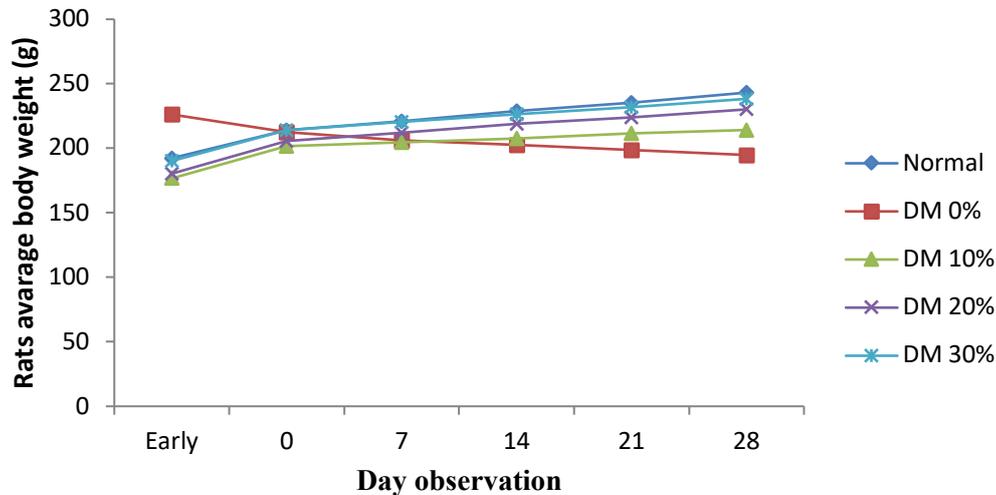


Figure 1 Body weight (average) of rats used in this study.

In this study, the blood sugar level of the DM rats fed with 0% instant porridge mix was 271.81 mg.dL⁻¹. We found that pumpkin and brown rice instant porridge mix significantly reduced blood sugar levels. In DM rats, the blood glucose levels were highest in DM rats fed with 0% instant porridge mix, followed by the 10%, 30%, and 20% treatments. The decrease in blood glucose level was because feeding DM rats with 10, 20, or 30% instant porridge mix reduced sugar levels in the blood compared to the 0%. Sharma and Rao [19] reported that pumpkin contains reducing sugar and total sugar of 77.30 and 90.13 mg.g⁻¹, respectively.

Table 2 The levels of blood insulin, glucose, and liver malondialdehyde in rats.

Treatment/rats group	Blood glucose levels (mg.dL ⁻¹)	Blood insulin levels (IU.dL ⁻¹)	Liver MDA levels (nmol.g ⁻¹)
Normal rats (standard feed)	89.97 ± 3.34 ^a	17.25 ± 0.06 ^e	0.97 ± 0.01 ^a
DM 0% (standard feed)	271.81 ± 10.76 ^e	5.94 ± 0.03 ^a	6.74 ± 0.03 ^e
DM 10%	146.37 ± 10.43 ^d	10.12 ± 0.05 ^b	4.76 ± 0.02 ^d
DM 20%	99.66 ± 1.84 ^b	14.31 ± 0.05 ^c	2.22 ± 0.02 ^c
DM 30%	111.93 ± 1.76 ^c	15.47 ± 0.11 ^d	1.41 ± 0.01 ^b

Note: values with the same letter within a column show no significant difference at $p < 0.05$ and vice versa.

The effects of polysaccharides in a pumpkin on rats with type 2 diabetes include increased insulin tolerance and HDL and decreased blood glucose, total cholesterol, and LDL [20]. Polysaccharides in pumpkin consist of xylose, arabinose, glucose, rhamnose, galactose, and glucuronic acid that reportedly exhibit good scavenging function against hydroxyl radicals and hydroxyl anions. In addition, polysaccharides in pumpkin function as antioxidants [21]. Treatment with chayote juice at a dose of 1 mL.100g⁻¹ of body weight of rats per day decreased the triglycerides, total cholesterol, and LDL and increased HDL [22]. Pumpkin porridge and arrowroot starch at a 5:1 had a resistant starch content of 11.97% [23], affecting blood glucose levels in rats because resistant starch is not easily digested into glucose.

Insulin levels of blood in rats

The insulin level of the blood in Table 2 shows that the DM rats fed with 0% instant porridge mix exhibited the lowest level of insulin (5.94 ± 0.03 IU.dL⁻¹). In contrast, DM rats fed with 10, 20, and 30% instant porridge mix had higher blood insulin levels. We found that the higher the substitution level of instant porridge mix, the higher the insulin levels in the blood of the DM rats. In contrast to the blood glucose level (except rats fed with 20% instant porridge mix), the higher the blood insulin, the lower the blood glucose. This further strengthens the notion that treatment with pumpkin and brown rice instant porridge mix can improve diabetes conditions by increasing insulin production. This study demonstrated a positive possibility of insulin production for DM rats.

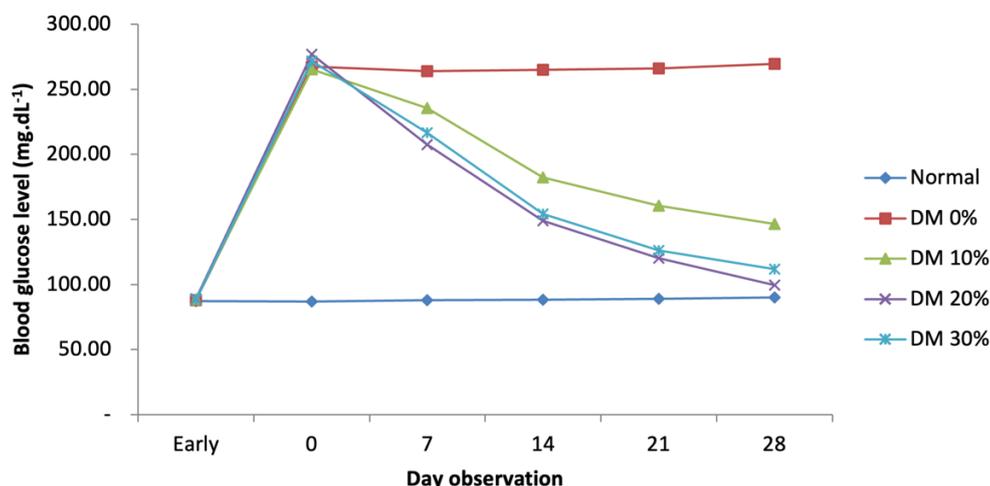


Figure 2 Rats blood glucose levels during the experiment.

Malondialdehyde (MDA) levels in rats

Diabetes is associated with oxidative stress. In the current study, STZ induction was used to trigger oxidative stress to cause diabetes in rats. It has been reported that oxidative stress can lead to type 2 diabetes, cell dysfunction, impaired glucose tolerance, and resistance to insulin [24]. The determination of malondialdehyde (MDA) in biological materials is widely used as an indicator of the presence of free radicals and oxidative damage, especially in unsaturated fatty acids that have more than one double bond. Malondialdehyde is an end-product of lipid peroxidation after free radicals attack the lipid membranes, which are rich in polyunsaturated fatty acids (PUFA). Analysis of free radicals was carried out by determining the levels of MDA in the liver of the rat (Table 2).

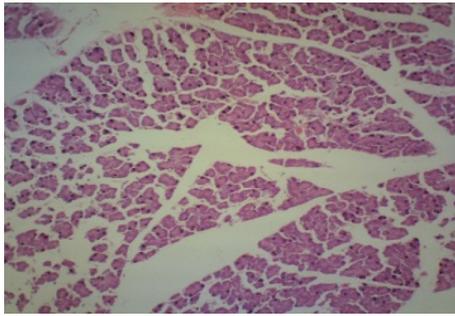
From Table 2, DM rats fed with 0% pumpkin and brown rice instant porridge mix had higher levels of MDA than the normal rats, and DM rats fed with 10, 20, and 30% instant porridge mix. Therefore, the treatment of DM rats with instant porridge mix can reduce MDA levels. The MDA levels in DM rats fed 30% instant porridge mix was 1.41 nmol.g^{-1} , and closer to the MDA level (0.97 nmol.g^{-1}) of rats fed on the standard feed, compared to DM rats fed with 10 and 20% instant porridge mix. Diabetes can increase the activity of fatty acyl-coenzyme A oxidase, which initiates the oxidation of fatty acids and results in lipid oxidation. STZ can trigger an increase in blood glucose and free radicals. Also, an increase in blood glucose levels can promote oxidative stress. Advanced glycation end products, peripheral nerve polyol, protein kinase activation, oxidative phosphorylation, and glucose automation pathways, which increase oxidative stress due to increased blood glucose levels.

Table 2 and Figure 2 show that the reduced blood glucose level in rats fed with instant porridge mix can decrease MDA level and increase blood insulin. The level of MDA in DM rats fed 10, 20, and 30% instant porridge mix were lower than that in 0% treatment. Animals, including rats, possess an internal oxidative stress defence system that can be increased by fortifying external antioxidants that are needed by the pancreas because it has a limited defence system against oxidative stress [25]. Pumpkin and brown rice instant porridge mix has high antioxidative activity and can function as an antidote against oxidative stress.

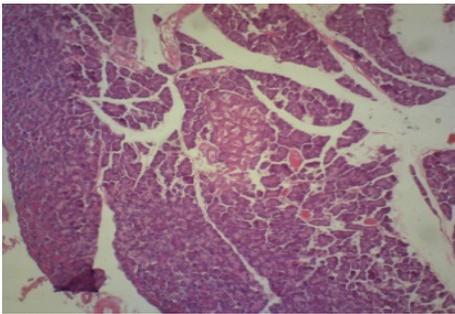
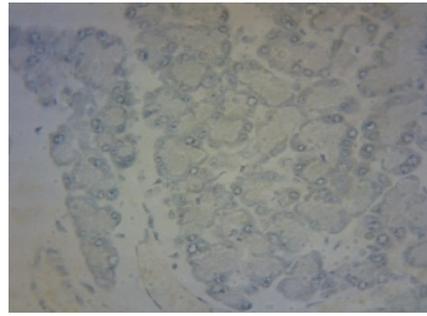
A higher ethyl acetate extract dose corresponds with the lower level of MDA in the liver of DM rats. Pumpkin and brown rice instant porridge contain 0.19% phenol [23] which acts as antioxidants, and therefore, the higher the dose, the greater the oxidative activity and the lower the MDA level. The antioxidant activity exhibited by phenol is associated with the balance of oxidation-reduction reaction. The electron is donated to the aromatic ring, thus increasing the speed of the oxidation inhibition reaction by antioxidants. Natural plant antioxidant compounds are generally phenolic compounds that are multifunctional and act as antioxidants because of their ability to act as reducers, free radical scavengers, metal binders, or triplet oxygen made from the singlet form [26], [27].

Histopathology of the pancreas

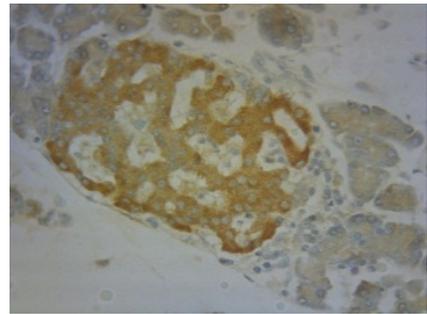
The histopathology results are presented in Figure 3A through 3E. Figure 3a is a histological picture of the pancreas for the control group (DM rat given standard feed or fed on 0% instant porridge mix). The results of hematoxylin-eosin (HE) staining in these samples showed swollen cells and purple color (but not intense brown color). The brownish color indicates the presence of β -pancreatic cells, while the purple ones are not β -pancreatic cells.



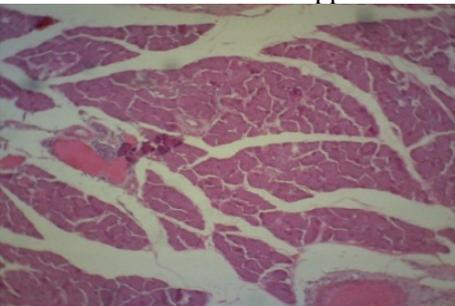
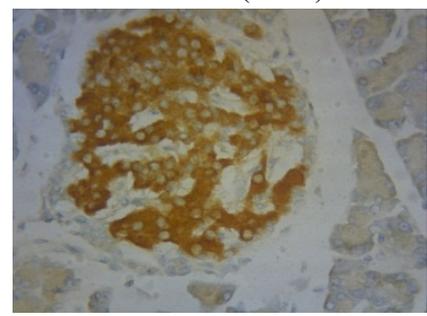
a. The appearance of islets of Langerhans in 0% DM rats (520X)



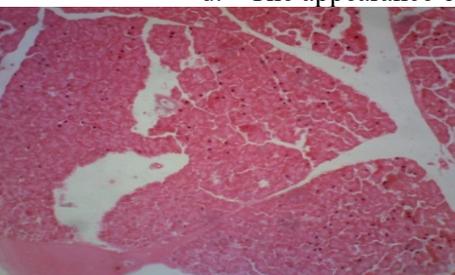
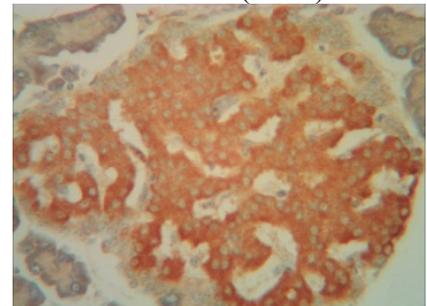
b. The appearance of islets of Langerhans in 10% DM rats (520X)



c. The appearance of islets of Langerhans in 20% DM rats (520X)



d. The appearance of islets of Langerhans in 30% DM rats (520X)



e. The appearance of islets of Langerhans in normal rats with standard feed (AIN 95) (520X)

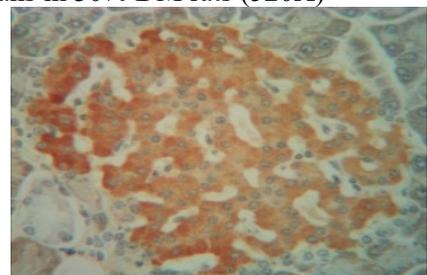


Figure 3 Histology of the pancreas stained with HE.

This is very different (Figure 3a) from the staining results in the normal rats (Figure 3e), which showed a high brown color intensity with a very dense nucleus. The brown color indicates that insulin production occurs in the pancreas. Also, the control rats showed no β -pancreatic cells but necrosis and degeneration of the islands of Langerhans. Necrosis is characterized by empty spaces in the middle of the islands of Langerhans. According to Szkudelski [16] and Rajeswari, Kesayan, and Jayakar [28], this observation supports the notion that STZ induction results in damage to β -pancreatic cells, decrease in the production of insulin, and an increase of glucose, all of which lead to hyperglycemia.

Based on the semi-quantitative observational data (Table 3), the intensity of insulin-positive cells in the control DM rats was negative (-), but in the DM rats fed 10, 20, and 30% instant porridge mix was increased intensity of insulin-positive cells. Feeding or treating DM rats with pumpkin and brown rice instant porridge mix can increase the intensity of insulin-positive cells, regenerate pancreatic beta cells and increase the ability of insulin secretion, as shown by the increased insulin and reduced glucose levels in the blood in this study.

Table 3 The intensity of insulin-positive cells in the islands of Langerhans in rats.

Parameter	Treatments				
	Normal rats (standard feed)	DM 0% (standard feed)	DM 10%	DM 20%	DM 30%
Insulin-positive cell intensity	+	-	++	+++	+++

Note: DM: diabetes mellitus; negative: -; weak intensity: +; moderate intensity: ++; high intensity: +++

CONCLUSION

Taken together, pumpkin and brown rice instant porridge mixed can reduce blood glucose and liver malondialdehyde levels while increasing blood insulin levels of diabetic rats. The higher the intake of instant porridge mix, the lower the blood glucose level and the less liver damage due to the reduced oxidative stress and higher insulin level in the blood. Feed substitution treatment at 20% was the best in reducing blood sugar and regeneration of pancreatic cells in diabetic rats.

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