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Glucan-rich polysaccharides obtained from split gill mushroom [*Schizophyllum commune* (Fr.)] ameliorate hyperglycemia by enhancing insulin and GLUT2 pancreas in type 2 diabetic rats

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Summary. Aims. Abnormalities in the secretion of insulin are the cause of pathology and complications in diabetic patients. The aim of this study was to investigate the anti-diabetic effect of polysaccharide extracts from the split gill mushroom in type 2 diabetes rats administered a low dose of streptozotocin (STZ) in combination with a high-fat diet.

Methods. The rats were divided into 6 groups: the control group (ND), the control group fed with polysaccharide extract from split gill (ND240), the diabetes group (HFD+DM), the diabetic group fed 120 (HFD+S120) and 240 mg/kg BW polysaccharide extract (HFD+S240), and the diabetic group receiving metformin (HFD+Met). Subsequently, the Islets of Langerhans of pancreatic tissue were studied using a light microscope and transmission electron microscopy (TEM). Immunofluorescence for the detection of insulin and glucose transporter 2 (GLUT2) proteins, and malondialdehyde (MDA) were also detected in pancreatic tissue.

Results. In the diabetic and HFD+120 groups, the tissues harbored various pathologies. The HFD+S240 and HFD+Met groups were found to have lower blood sugar levels. The levels of insulin and GLUT2 increased compared with the diabetic group. Additionally, the levels of MDA were reduced.

Conclusions. The use of polysaccharide extract from split gill mushrooms (240 mg/kg BW) is an alternative to treating various pathologies in the relief or treatment of diabetes mellitus.

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Introduction

As a result of faulty pancreatic function, diabetes, also known as diabetes mellitus, is a chronic and hereditary disease that affects a significant number of individuals today. The pancreas plays a critical role in the regulation of blood glucose levels, and its improper functioning is indeed a key factor in diabetes. The pancreatic beta (β) cells are responsible for the production of the hormone insulin, which is either very low or nearly nonexistent. The levels of sugar in the blood can be better managed with the use of this hormone. The condition known as hyperglycemia, which is characterized by extremely high blood sugar levels, is brought on by abnormalities in the secretion or activity of the insulin hormone. When it comes to diabetic individuals, hyperglycemia is the primary factor that leads to clinical problems and pathology. These kinds of difficulties lead to the deterioration of the structure and function of critical organs and systems in the body, particularly the liver and the pancreas.

β cells in the pancreas are responsible for the production of insulin, which is called pre-proinsulin. It is then stored in granules after being converted to proinsulin by the β cells, which are in the endoplasmic reticulum (ER). With the help of the granules, the C peptide can convert proinsulin into insulin by cleaving it. The levels of glucose in the blood govern the release of β-insulin. When the level of glucose in the blood is higher than 70 mg/dL, the translation process is accelerated, which subsequently stimulates the production of insulin. After that, glucose is

phosphorylated by hexokinase to produce glucose-6-phosphate (Berger and Zdzieblo, 2020) when it enters the pancreatic β cell through the GLUT2 pathway. Afterward, ATP is introduced into the mitochondria by means of a variety of metabolites. In β cells, an increase in ATP causes the ATP-sensitive K^+ channel to close, which in turn causes membrane depolarization. Additionally, the voltage-gated calcium channel opens, which enables calcium to enter the cell. Because of this, exocytosis occurs, which causes insulin to be released. When insulin attaches to the receptor, it triggers the activation of the enzyme tyrosine kinase, which in turn triggers several signal transductions, ultimately resulting in the absorption of glucose transporter 4 (GLUT4) into the cell membrane (Fridlyand and Philipson, 2010). A greater amount of glucose is taken in by the cells because of this. It is the joint effect of insulin that causes an increase in the storage of carbohydrates in the form of glycogen, as well as an increase in the synthesis of fat and protein within the cells.

Some of the most nutrient-dense foods are mushrooms. There are numerous advantages for the body; one of them is that it helps to strengthen the immune system. Split gill, also known as *Schizophyllum commune* (Fr.), is a local mushroom and culinary item in the area. The consumption of this substance has been documented in a multitude of nations, including India, Malaysia, Nigeria, Russia (9-11), and Southeast Asian countries, including Thailand, Laos, and Myanmar (Yim et al., 2013). In the current investigation, the crude polysaccharide of split gill was found to be composed of carbs (65.8%), proteins (13.4%), and sulfates (3.29%) (Yelithao et al., 2019). There is a high fiber content, a high protein content, and a low fat content, which are all significant nutrients and phenolic compounds. The cell wall is made up of β-glucans that are cross-linked with chitin at various points. The polysaccharide, comprising β-glucan, can be seen in the cell wall. The β-glucan molecule has a considerable amount of antioxidant action. In the mushroom, the β-glucans, also known as schizophyllan, have a structure that resembles a triple-stranded helix (Leung et al., 2006). Schizophyllan is a polymer composed of β-1,3-D-glucans that also contains β-1,6-D-branches, and possesses a high phenolic content (Lee et al., 2020). It plays a significant role when it comes to combating free radicals, which are responsible for oxidative damage. It possesses several medical qualities, including anti-cancer, anti-tumor, anti-microbial, anti-viral, and antioxidant properties (Saetang et al., 2023). However, even though the split gill mushroom is a local mushroom that offers a multitude of advantages, most people are still not knowledgeable about the advantages of such mushrooms. In addition, researchers have found additional compounds that are significant. It was observed that the ethyl acetate extract of split gill mushrooms, which contains phenolics and terpenoids, results in a reduction in the level of glucose in the blood, an improvement in the lipid profile, and level of antioxidant parameters (Sharma et al., 2021). To this day, there is no information available regarding the effectiveness of polysaccharide extracts from split gill mushrooms when used as a supplement for the treatment of diabetes. The objective of this study was to investigate the effects of polysaccharide extracts on antidiabetic activity, specifically what effect they have on lowering blood sugar levels and their effect on pancreatic tissue in rat models of type II diabetes. In addition to influencing alterations in the ultrastructure of pancreatic β cells and tissues of the islets of Langerhans in the pancreas, the expression of proteins that play a role in the uptake of glucose into the tissues, including insulin and GLUT 2, was investigated using a diabetic rat model.

Materials and methods

Eight-week-old male Wistar rats were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand, and used in this study. We kept and maintained all animals in a controlled animal laboratory environment with an alternate 12-hour light/dark cycle (25±2°C), fed them with standard rat chow, and provided them with unlimited access to water. The Institutional Animal Care and Use Committee of the Prince of Songkla University (MHESI 68014/791, Ref. ARO25/2022) approved and guided the experimental protocols described in this study. For the purpose of this research, 60 male Wistar rats (n=10) aged 8 weeks were used and divided into six groups: a normal control group (ND), a group fed with daily polysaccharide extract from split gill mushrooms (240 mg/kg of body weight) (ND240) in addition to normal food intake, a high-fat diet group (HFD+DM) (DIO rodent purified diet with 60% energy from fat-blue 58Y1, Test Diet®, Richmond, IN, USA), a high-fat diet group with diabetes that received the polysaccharide extract from split gill mushrooms 120 and 240 mg/kg of body weight (HFD+S120 and HFD+240, respectively), and a high-fat diet group with diabetes treated with metformin (20 mg/Kg BW) daily by oral ingestion (HFD+Met). Diabetes was induced in the animals with an injection of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) (35 mg/kg BW) dissolved in 0.1 mol/l citrate buffer by intraperitoneal injection. Control rats received an injection with citrate buffer alone. Over the next week, we drew blood from the tip of the tail to measure blood sugar levels. The blood sugar levels were measured and analyzed by a one-touch glucometer (Accu-Check Active® and test strips, Roche Diagnostics, Mannheim, Germany).

Extracting crude polysaccharides from the split gill mushroom

Methods for extracting crude polysaccharides from the split gill mushroom were modified from a protocol previously described (Sermwittayawong et al., 2018). In brief, the gill mushroom was washed, shredded, dried at

70°C for 48 hours, and ground into powder with a grinder. A mixture of 8 ml of water and 1 g of ground mushroom was boiled at 90-95°C for 4 hours. After cooling to room temperature, we centrifuged the mixture at 5,000 rpm for 10 minutes. The researchers subjected the residue to another round of boiling after collecting the supernatant. The supernatant from the two rounds of extraction was pooled, and the volume of the supernatant was reduced using a rotary evaporator (60°C, 100 rpm, 100 mbar, and cooling temperature 10°C). Then, the polysaccharide in the sample was precipitated by adding 3 volumes of absolute ethanol, followed by incubation at 4°C for 12-16 hours. Centrifugation separated the precipitated polysaccharide from the supernatant. The precipitates were pooled and dissolved in distilled water. Subsequently, the remaining ethanol in the polysaccharide solution was eliminated by dialysis with distilled water. The dialyzed sample was subjected to lyophilization and was kept in a desiccator until needed (Sermwittayawong et al., 2018).

The quantification of total glucan (comprising α-glucan and β-glucan) along with D-glucose in oligosaccharides, sucrose, and free D-glucose was performed. The determination of β-glucan levels in split gill mushrooms utilized the Mega-Calc™ method from Megazyme. The calculation for β-glucan content involved subtracting α-glucan from the total glucan amount.

Lipid peroxidation (MDA) assay

An indicator of oxidative stress in pancreatic tissues was measured using the Thiobarbituric Acid Reactive Substances (TBARS) by the Lipid Peroxidation (MDA) Assay Kit (Sigma-Aldrich, St. Louis, MO, USA, Cat. No. MAK085) (Yuksel et al., 2016) at a wavelength of 532 nm. The tissues were prepared, and the proteins were extracted in a lysis solution and centrifuged at 13,000 x g for 10 minutes to collect the supernatant. MDA standards were prepared at concentrations of 20-100 µM. A total of 200 µl of samples and standards were placed into the wells containing 600 µl of TBA reagent, incubated at 95°C for 60 min, and then cooled on ice for 10 min before absorbance measurement. The results were reported as nmol/mg of tissue.

Hematoxylin and Eosin (H&E) and Masson's trichrome staining

All pancreas tissues from all groups were fixed in 10% formalin and dehydrated in a graded series of ethanol (70%, 80%, 90%, 95%, and 100%), with two changes each for 1 hour. After this, three changes of xylene for 30 minutes each were used as clearing reagents before the tissue sections were embedded in paraffin, cross-sectioned at 5 µm thickness and stained with H&E and Masson's trichrome staining methods. The sections were then examined and photographed under an Olympus light microscope (BX-50, Olympus, Japan).

Transmission electron microscopy technique

Small pieces of pancreatic tissue (1 mm^3 in size) were taken and immediately fixed in 2.5% buffered glutaraldehyde. The tissues were then post-fixed in 1% osmium tetroxide, dehydrated, infiltrated with propylene oxide, and embedded in resin. Semi-thin sections (about 0.5 µm or 1.0 µm) stained with Toluidine blue served as a guideline for the area of interest and were further trimmed. For ultrathin sections (about 60 nm), an ultramicrotome was used to cut them, and they were subsequently stained with uranyl acetate and lead citrate solutions. These ultrathin sections were spread mostly on 200 or 300 mesh. The sections were examined and photographed under a transmission electron microscope (TEM-JEM2010, JEOL, Japan).

Immunofluorescence study

To determine the levels of insulin and GLUT2, the cut pancreatic tissues were deparaffinized in xylene, hydrated through a descending series of ethanol to distilled water, and permeabilized in PBS with 0.1 Triton X-100 (PBST) for 30 min. Blocking was performed using horse serum in PBS for 1 h at room temperature, followed by incubation with rabbit monoclonal anti-insulin antibody together with rabbit anti-GLUT2 (Abcam, Cambridge, UK) diluted 1:100 in blocking serum at 4°C overnight. After washing three times with PBS, the sections were exposed to Texas red goat anti-rabbit IgG (H+L) antibody (1:200; Vector Laboratories, Inc.) in blocking solution to detect insulin and GLUT2, respectively, for 2 hours at room temperature in the dark. We examined the images under a fluorescence microscope (BX-50, Olympus Corporation). The percentages of insulin and GLUT2 cell expression were determined using National Institutes of Health (NIH) Image J software 1.52 to measure the fluorescence intensity (Komolkriengkrai et al., 2022).

Statistical analysis

The results were reported using the mean value, with the standard error of the mean represented as a range. The statistical methodology used in this study involved conducting an analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test. A *p*-value that falls below the predetermined threshold of 0.05 is statistically significant.

Results

Analysis results of β-glucan levels from polysaccharides with megazyme

The β-glucan levels were measured by the Megazyme method as follows; the gel extracted from β-

glucan of split gill mushrooms was analyzed using the Mega-CalcTM Yeast & Mushroom B-Glucan (KYBGL) Determination from Megazyme. The result showed that β-glucan levels in split gill mushroom gel was 43.04% w/w. (Table 1).

Blood sugar levels of rats.

For a period of eight weeks, we monitored the blood sugar levels in each group of rats weekly. When comparing the blood sugar levels of the HFD+DM and HFD+S120 groups against those of the ND and ND240 groups, we found that the HFD+DM, HFD+S120, HFD+S240, and HFD+Met groups had significantly higher blood sugar levels than the ND and ND240 groups (p<0.0001). Both HFD+S240 and HFD+Met were found to have a substantial impact on lowering blood sugar levels. However, the HFD+S240 exhibited a significantly lower blood sugar level (p<0.05) at weeks 6-8. The HFD+Met group exhibited a significantly lower blood sugar level (p<0.001) at week 6 and weeks 7-8 (p<0.0001) (Fig. 1).

Table 1. Glucan content in powdered split gill mushroom.

Total glucans (g/100 g dry mass)	α- glucans (g/100 g dry mass)	β- glucans (g/100 g dry mass)
43.52 (100%)	0.48 (1.10%)	43.04 (98.90%)

Malondialdehyde (MDA) detection method in pancreatic tissue

The influence that the presence of MDA has on the tissue of the pancreas is seen in Fig. 2. According to the findings, the levels of MDA in pancreatic tissue were determined to be 2.12±0.45 µmol/mg protein in the ND group and 1.63±0.38 µmol/mg protein in the ND240 group. According to the results of the trials, the control group given polysaccharide extract from split gill (ND240) did not result in an increase in the levels of MDA in the tissues of the pancreas. The MDA levels in pancreatic tissue were considerably higher (p<0.05) in rats with type 2 diabetes mellitus (HFD+DM) and HFD+S120 rats, being 4.133±0.30 and 3.96±0.46 µmol/mg protein, respectively, compared with rats in the ND and ND240 groups. Rats that were fed HFD+S240 and HFD+Met showed a decrease in MDA levels; specifically, the values obtained were 2.27±0.37 µmol/mg protein and 3.67±0.33 µmol/mg protein, respectively. Interestingly, when compared with diabetic rats, the group that consumed a high-fat diet and S240 demonstrated a drop in MDA levels in pancreatic tissue that was statistically significant (p<0.05). The MDA in the control group was comparable to this.

Histological study by the light microscopy technique

After 8 weeks, the pancreas tissues were excised to study the pathological characteristics under a light microscope at various magnifications. In the ND group,

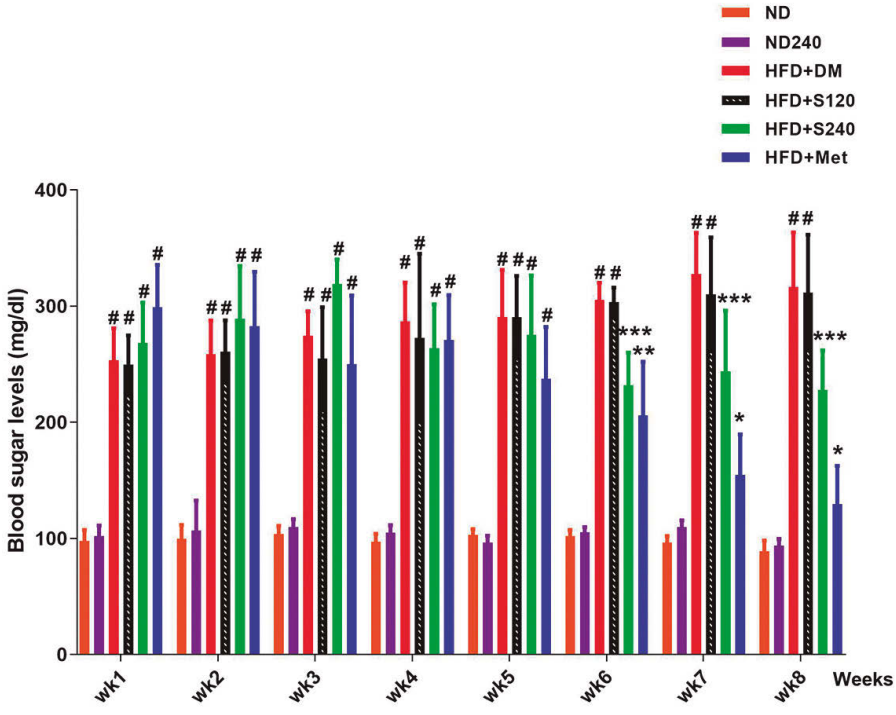

Fig. 1. Blood sugar levels of ND, ND240, HFD+DM, HFD+S120, HFD+S240, and HFD+Met groups for a period of 8 weeks. Values are mean±SE, $^{\#}p$<0.00001 in HFD+DM, HFD+S120, HFD+S240, and HFD+Met compared with ND rats. $^{***}p$<0.05 in HFD+SD240, $^{**}p$<0.001 and $^{*}p$<0.0001 in HFD+Met compared with HFD+DM rats.

there was no pathological alterations in the pancreatic tissue (Figs. 3A, 4A) nor the Islets of Langerhans. The ND240 group, which was not induced for diabetes but received polysaccharide extract from split gill at a concentration of 240 mg/kg BW, did not exhibit the pathology of cells and tissue in the area of the Islets of Langerhans (Figs. 3B, 4B). In contrast, the HFD+DM (Figs. 3C, 4C) and HFD+S120 (Figs. 3D, 4D) groups showed changes in the diameter and shape of the Islets of Langerhans. The cells in the Islets of Langerhans were wilted and atrophied, appearing to wither, and were much smaller than those in the ND and ND240 groups. Masson's trichrome staining showed that collagen fibers, evidenced by blue staining, were more congested than other groups (Fig. 4C). In the HFD+S240 group, the diameter of the Islets of Langerhans was improved. In addition, there was a decrease in cell damage in the Islets of Langerhans and cell regeneration (Figs. 3E, 4E). In the HFD+Met group, there was cell recovery in the Islets of Langerhans and a change in the diameter and shape of the Islets of Langerhans similar to that of the control group. The damage to cells in the Islets of Langerhans was also reduced (Figs. 3F, 4F).

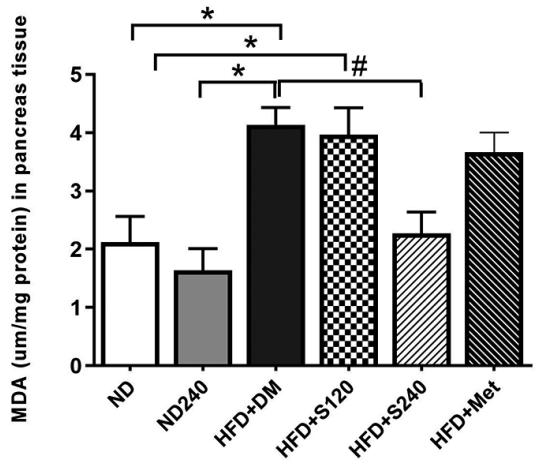

Fig. 2. Antioxidant capacities of pancreas tissue homogenate from ND, ND240, HFD+DM, HFD+S120, HFD+S240, and HFD+Met groups at 8 weeks. The MDA levels in HFD+DM rats. *p<0.05 compared with ND and ND240 rats. #p<0.05 compared with HFD+DM rats.

Histological changes in β cells of Islets of Langerhans by Transmission electron microscope (TEM)

The structure of β cells in the pancreatic islets of Langerhans was studied at the transmission electron microscopic level (Fig. 5). In the ND (Fig. 5A) and ND240 (Fig. 5B) groups, they had the appearance of cell organelles, a round nucleus (N), and a normal cytoplasmic organelle structure. In contrast, in the

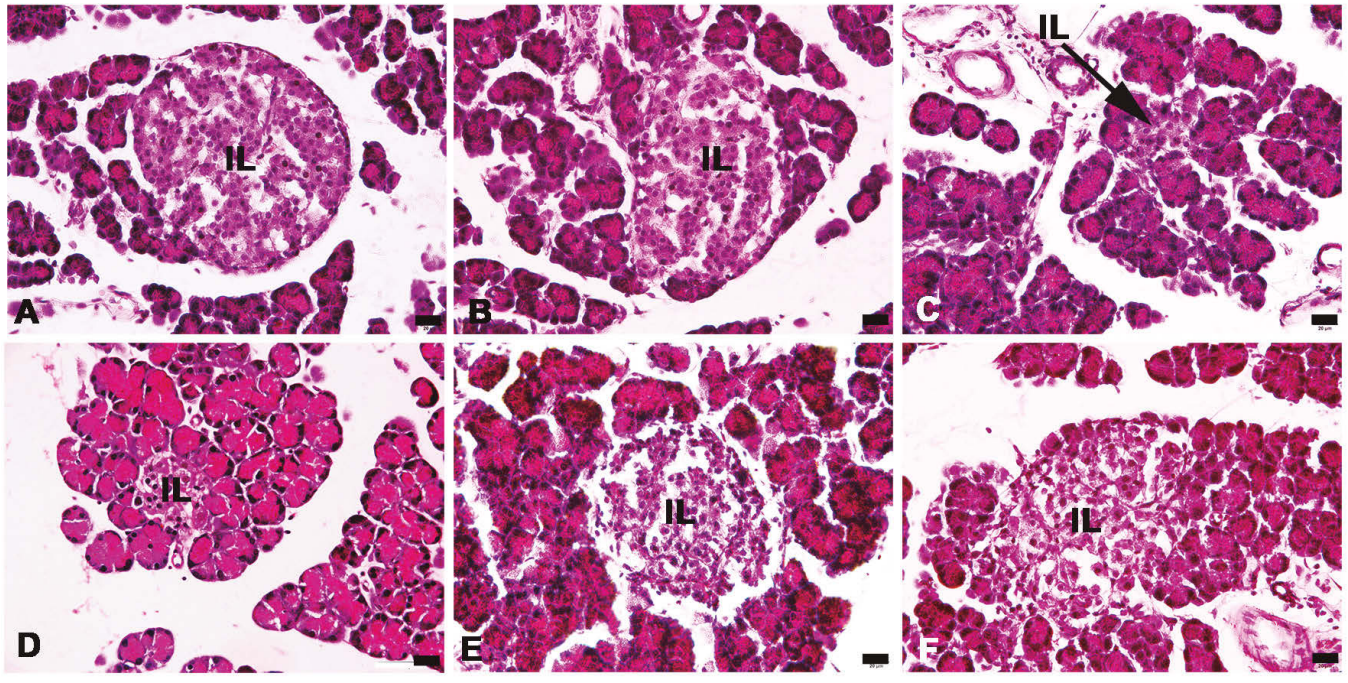

Fig. 3. Light micrograph showing H&E staining of pancreatic tissue in the Islets of Langerhans area (IL) in control rats (ND) **(A)**, control group receiving polysaccharide extract from split gill mushroom (ND240) **(B)**, diabetic group (HFD+DM) **(C)**, diabetic group that received polysaccharide extract from mushroom (HFD+S120) **(D)** and (HFD+S240) **(E)**, and diabetic group that received metformin (HFD+Met) **(F)**. When looking at the HFD+DM group, it was discovered that wilting and atrophy, as well as changes in the width and form of the Islets of Langerhans, had occurred. In addition, it causes damage to the cells in the Islets of Langerhans, which appear to be dying and are significantly smaller. Scale bar: 20 μm.

diabetic group (HFD+DM), the pathology showing cell apoptosis was found, i.e., cell atrophy and shrinkage, loss of membrane integrity, chromatin condensation, and degeneration of mitochondria. Only small amounts of microvesicular insulin granules were observed (Fig. 5C). In HFD+S120 rats, the pathology still showed degeneration of mitochondria and small amounts of microvesicular insulin granules were observed (Fig. 5D). The characteristics of the nuclei and organelles in the cytoplasm of β cells were restored in HFD+S240 rats (Fig. 5E), as in the HFD+Met group (Fig. 5F). However, the HFD+S240 group still exhibited some swollen mitochondria and massive vacuole overload.

Study of insulin and GLUT2 expression in the Islets of Langerhans by the immunofluorescence method

The expression of insulin and GLUT2 proteins in the Islets of Langerhans of ND (Fig. 6A) and ND+S240 (Fig. 6B) rats show the characteristic of strong red fluorescence and dense clusters in the Islets of Langerhans. A comparative study of insulin expression at the Islets of Langerhans found that the diabetic group (HFD+DM) had less insulin protein expression. This was characterized by low and sparse Texas red fluorescence (Fig. 6C) and had a significant difference (p<0.001) compared with the control (ND) group and the control group receiving polysaccharide extract (HFD+ND240) (Fig. 8A). In studying GLUT2 expression at the Islets of Langerhans (Fig. 7), we found that the HFD+DM group had low GLUT2 expression, exhibiting a characteristic red fluorescence. It was less and fainter (Fig. 7C) compared with the ND (Fig. 7A) and ND240 rats (Fig. 7B) and showed a significant difference (p<0.05) compared with the ND and ND240 groups (Fig. 8B). The HFD+N120 group (Fig. 7D) still had less GLUT2 protein expression, the same as the HFD+DM group.

In the group induced with type 2 diabetes mellitus that received 240 mg/kg BW (HFD+N240) of mushroom polysaccharide extract (Fig. 7E) and metformin (Fig. 7F), there was an increase in insulin (Fig. 8B) and GLUT2 protein expression. There was a significantly different (p<0.05) and more noticeable red fluorescence in insulin protein expression compared with the diabetic group (HFD+DM).

Discussion

This experimental model of type 2 diabetes was made by feeding the animals a high-fat diet and administering a low-dose streptozotocin (STZ) injection in male rats. Injecting STZ into rats increased their blood sugar levels as STZ, the most prominent diabetogenic chemical, is widely used in experimental animals for creating animal models of type 1 and type 2 diabetes (Ghasemi et al., 2014). Male Wistar rats for diabetes type 2 were fed a high-fat diet (HFD), which contained 61.6% fat, 20.3% carbohydrates, 18.1% protein, and 5.12 kcal/g of energy. When feeding foods that contain

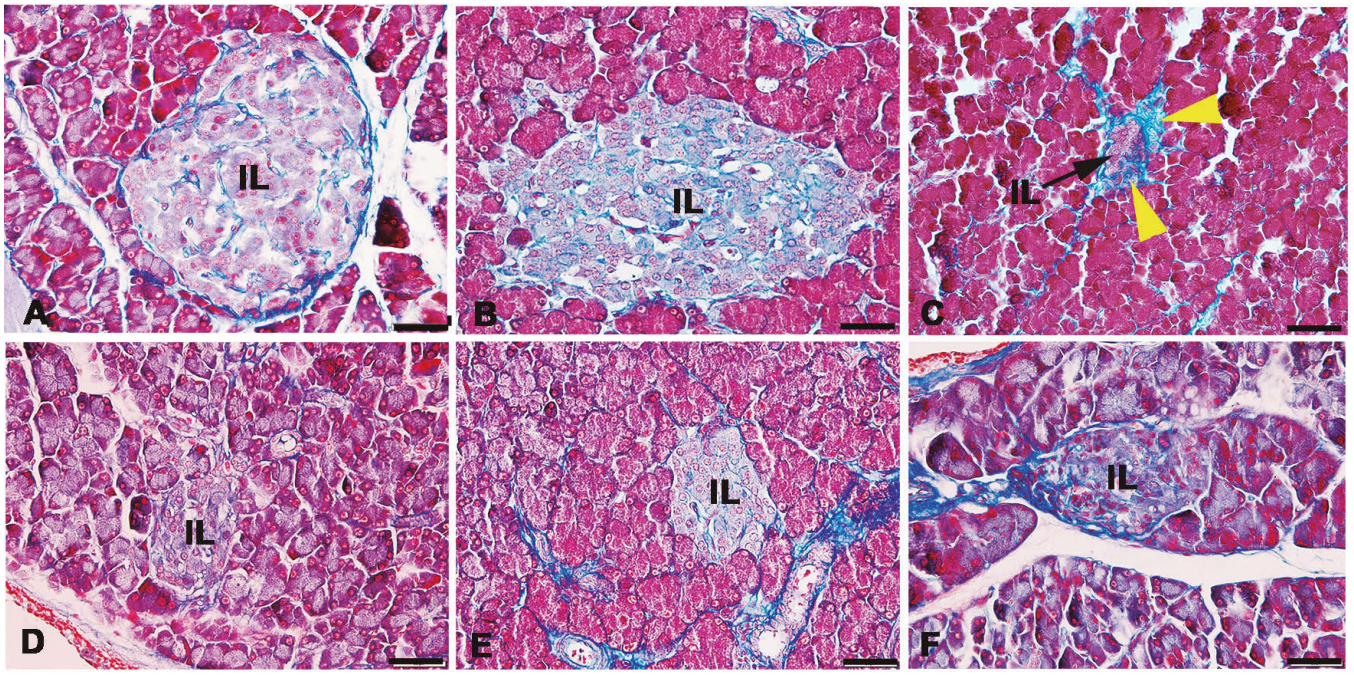

Fig. 4. Light micrograph showing Masson's trichrome staining of pancreatic tissue in the Islets of Langerhans area (IL) in control rats (ND) **(A)**, ND240 **(B)**, HFD+DM **(C)**, HFD+S120 **(D)**, HFD+S240 **(E)**, and HFD+Met **(F)** groups. Collagen fibers were found to be accumulating all over the islets of Langerhans when the tissues were stained blue (yellow arrowheads). This was notably evident in the group that had been given a high-fat diet and diabetes **(E)**. Scale bar: 20 μm.

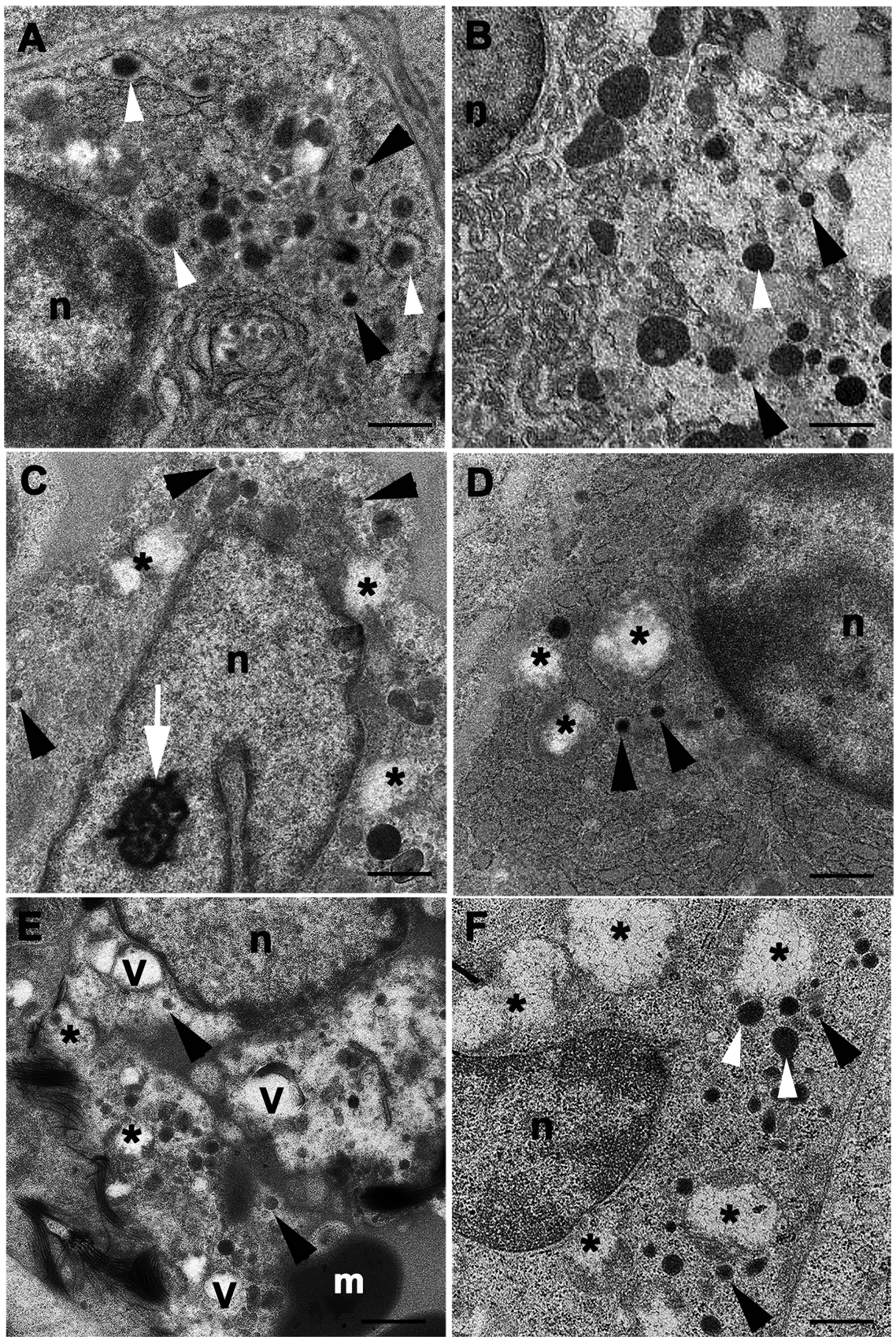

Fig. 5. Transmission electron micrograph comparing the appearance of β cells in the islets of Langerhans in the pancreas from each group: ND **(A)**, ND240 **(B)**, HFD+DM **(C)**, HFD+S240 **(D)**, and HFD+Met **(E)**. All groups show intracellular organelles, including the nucleus (n), and structures in the cytoplasm, including insulin granules, both macrovesicle (white arrowheads) and microvesicle granules (black arrowheads). Pathology in the diabetic group indicates cells entering apoptosis are characterized by chromatin condensation (white arrow), degeneration of mitochondria (black asterisk), and small amounts of microvesicular insulin granules. In addition, in the HFD+S240 group, massive vacuole overload (v) and swelling mitochondria (m) were also found. Magnification 11000x. Scale bar: 1 μm.

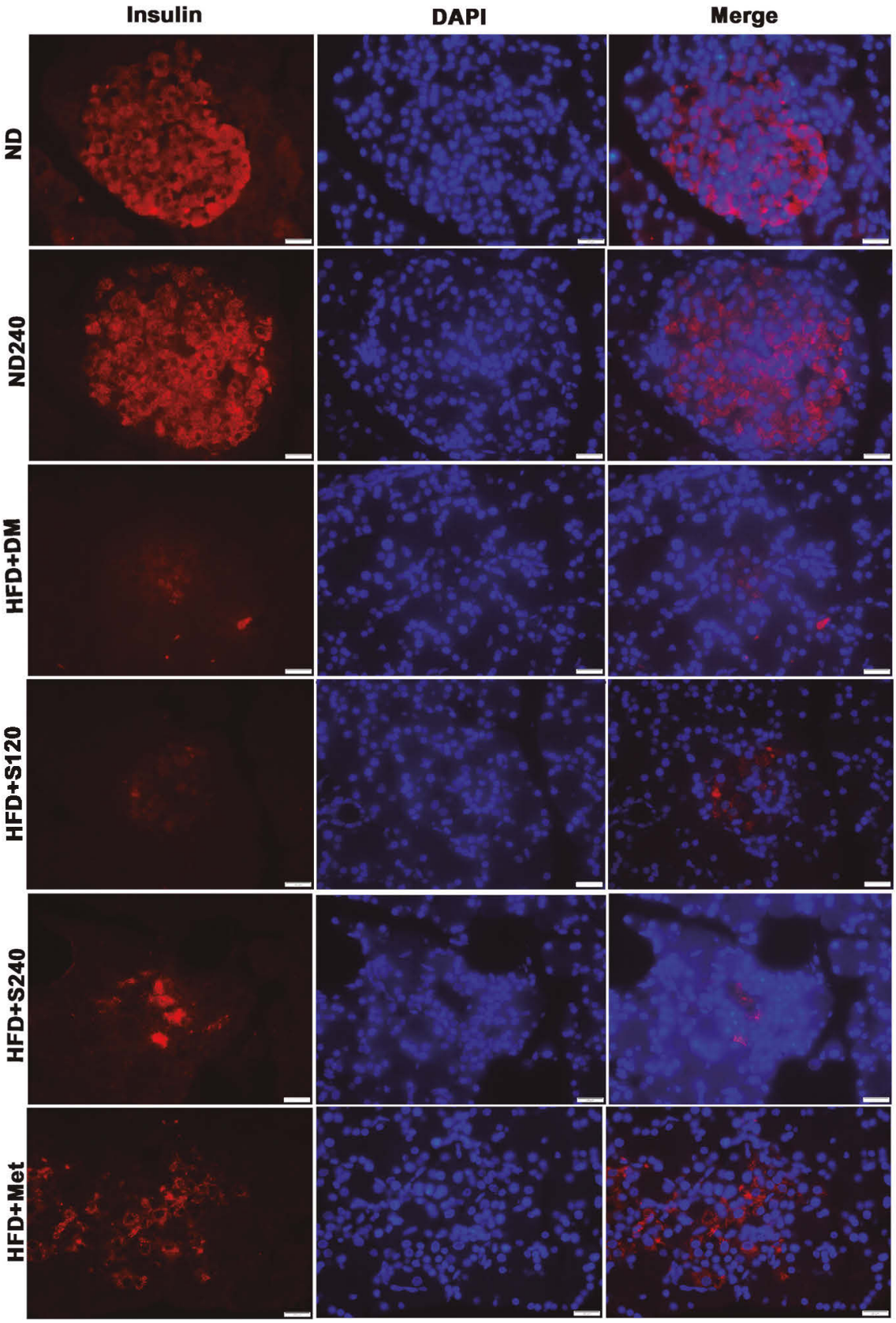

Fig. 6.
Photomicrographs of the insulin immunofluorescence reaction in the islets of Langerhans of pancreatic tissue from each group: control (ND), ND240, diabetic (HFD+DM), (HFD+S120), (HFD+S240) and HFD+Met. Scale bar: 20 μm.

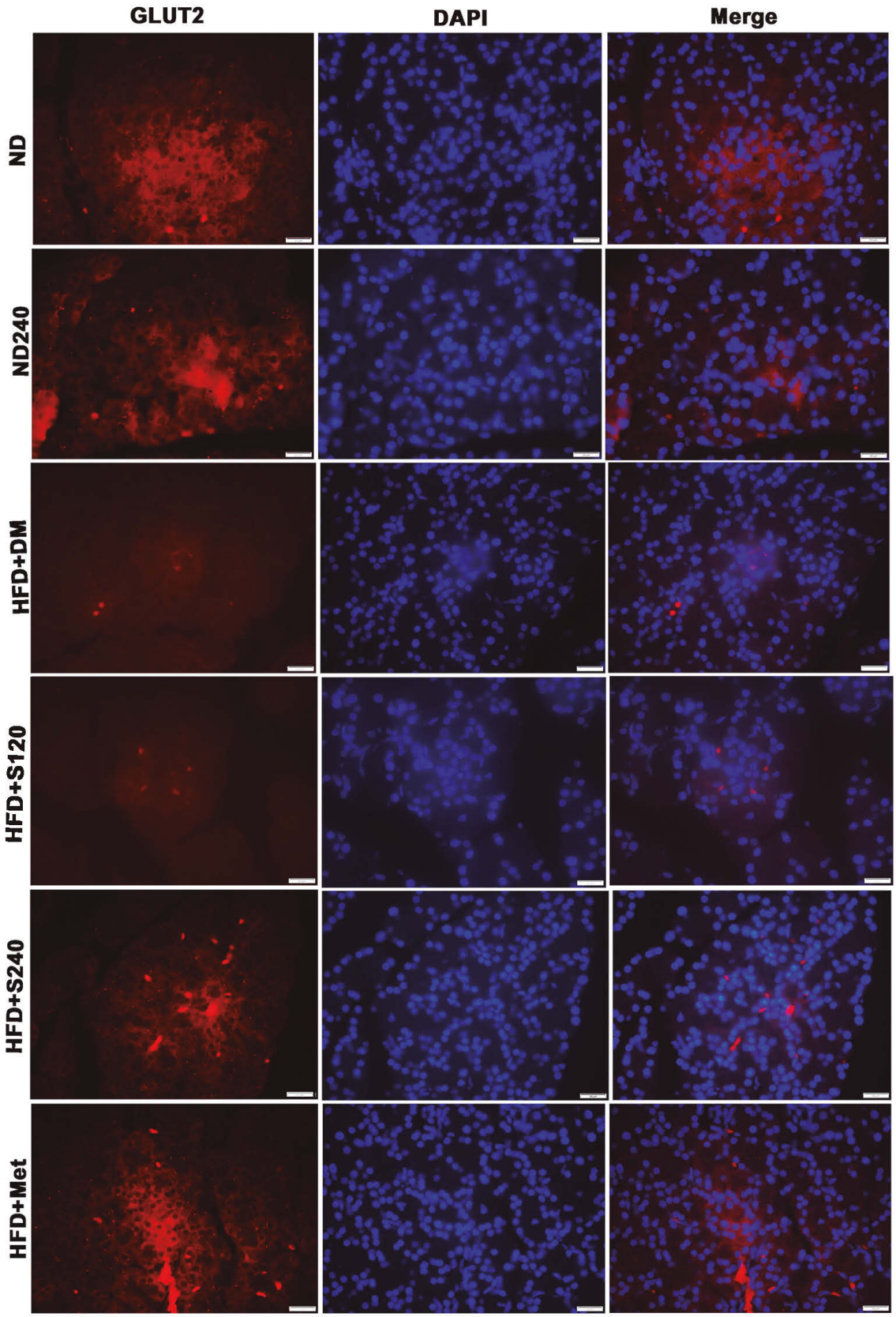

Fig. 7.
Photomicrographs of the GLUT2 immunofluorescence reaction in the islets of Langerhans of pancreatic tissue from each group: control (ND), ND240, diabetic (HFD+DM), (HFD+S120), (HFD+S240) and HFD+Met. Scale bar: 20 μm.

fat and carbohydrates in quantity and are low in dietary fiber for a period of 3 months, rats became obese and resistant to insulin resistance. STZ is a highly selective pancreatic islet β cell cytotoxic agent that is responsible for transporting the native molecule across cell membranes, which can cause β-cell damage. Pancreatic β cells produce and release the hormone insulin. Malfunctioning β cells causing impaired insulin secretion significantly contribute to the onset of high blood sugar levels (hyperglycemia) (Kahn, et al., 2006). The mechanism by which STZ contributes to diabetes has the same mechanism of entry into the cell as the release of glucose into the cell by a protein called glucose receptor transporter 2 (GLUT2). When the blood sugar level is higher, it will affect the induction of mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) activation. Continuous stimulation due to high glucose levels (hyperglycemia) triggers the long-term activation of PKC, an enzyme family crucial for regulating other protein functions (Lien et al., 2021). PKC has been linked to various vascular changes, including heightened permeability, increased contractility, synthesis of the extracellular matrix, cellular growth, apoptosis, angiogenesis, leukocyte adhesion, and modulation of cytokine activity.

In this study, when comparing the tissue characteristics of the pancreas from histological studies, we found that the diabetic group (HFD+DM) had tissue-dependent pathology that differed from the ND and ND240 groups. The Islets of Langerhans were wilted and atrophied, and the diameter, shape, and accumulation of collagen fibers in the Islets of Langerhans were altered. The damaged Islets of Langerhans appear to wither and are much smaller. When cells become less responsive and resistant to insulin, they cannot effectively take in glucose for energy production. This causes a deficiency of energy within the cells, which can lead to apoptosis.

From the TEM study, the pathology of the HFD+DM group indicated that cells entering apoptosis are characterized by cell atrophy and shrinkage, loss of membrane integrity, chromatin condensation, and degeneration of mitochondria. The cellular changes observed in TEM are the result of a complex interplay of factors such as insulin resistance, oxidative stress, mitochondrial dysfunction, inflammation, ER stress, and advanced glycation end products (AGEs) formation. These factors contribute to cellular damage and ultimately lead to the loss of cell integrity and function, consistent with hyperglycemia, which leads to inflammation and slows down blood flow to supply cells and tissues (Yuan et al., 2019).

High blood sugar accelerates atherosclerosis when the arteries become stiff and contain large amounts of cholesterol fat that obstruct the blood flow and deteriorate blood vessels. In diabetic rats, the Islets of Langerhans in the pancreas showed significant atrophy. Many damaged cells were found within the Islets of Langerhans, especially β cells, preventing the normal production of insulin. This is in line with research stating that, in diabetic patients, the Islets of Langerhans in the pancreas will be atrophied (Xin et al., 2017), unable to function, and β cells will decrease significantly. In pancreatic β cells, GLUT2 is needed for glucose-stimulated insulin secretion. Increased uptake of glucose from the bloodstream into pancreatic cells via GLUT2, where glucose is phosphorylated by hexokinase to glucose-6-phosphate (Thorens, 2015). Then, through various metabolites, ATP enters the mitochondria. When the ATP in β cells increases, the ATP-sensitive K^+ channel is closed, causing membrane depolarization and the voltage-gated calcium channel to open, allowing calcium to flow into the cell. This causes exocytosis to release insulin into the bloodstream (Tarasov et al., 2004). In this immunofluorescence study, both insulin and GLUT2 are proteins involved in glucose metabolism. The increase in insulin and GLUT2 expression suggests improved glucose utilization and transport in response to the treatment. Polysaccharides from split gill mushrooms influenced gene expression patterns, leading to the upregulation of the proteins.

When the body has high blood sugar levels, the formation of AGEs when glucose binds to intracellular proteins (non-enzymatic glycation) converts them into

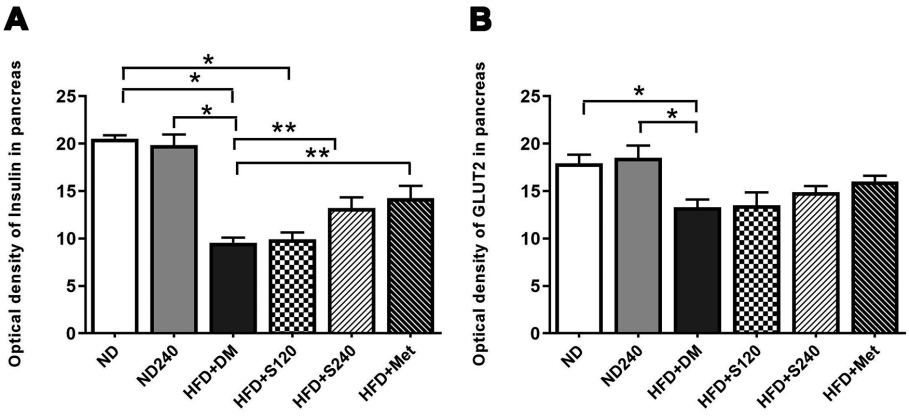

Fig. 8. The relative insulin optical density in the pancreas was analyzed. Values are mean + SE, *p<0.001 in DM groups when compared with ND and ND240, *p<0.05 in the HFD+DM group when compared with HFD+S240 and HFD+Met groups. **B.** The relative GLUT2 optical density in the pancreas was analyzed. Values are mean±SE, *p<0.05 in the HFD+DM group when compared with ND and ND240 groups.

AGEs through blood vessels and adds more free radicals (Twarda-Clapa et al., 2022). It causes thickening of the basement membrane with the addition of type 1 and type 4 collagens, causing the loss of the elasticity of the blood vessels. This affects the activity of endothelial nitric oxide synthase (eNOS) and guanylyl cyclase, resulting in reduced blood vessel response to nitric oxide (NO), divided into enzyme-independent and enzyme-dependent free radical formation. This includes an increase in PKC (Tran et al., 2022), a protein involved in intracellular signaling stimulated by diacylglycerol (DAG), which is produced by glycolysis in hyperglycemia, which activates PKC-β such as vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β). NO levels and reactive oxygen species (ROS) are induced by NADPH oxidase, which results in vascular endothelial dysfunction in diabetes (Murugan et al., 2020).

Polysaccharides are complex carbohydrates composed of monosaccharide units and linked by glycosidic bonds. Divided by monosaccharide composition into homopolysaccharides and heteropolysaccharides, both polysaccharides and monosaccharides can be linked linearly or separated into complexes. Previous studies have shown that bioactive polysaccharides are prevalent among fungi. β-D-glucan is a type of dietary fiber found in various sources, such as cereals, grains, yeast, mushrooms, and prebiotics. Effective for preventing and managing obesity, cardiovascular disease, diabetes, and cancer (Chen and Seviour, 2007; Ahmad et al., 2010). In addition to the fact that β-glucan increases hyperglycemia, it has been shown that administration of β-glucan under diabetic conditions improves systemic function and resistance to diabetic complications (Mihailović et al., 2013). β-glucan is one of the main active components of mushrooms and is found in a variety of mushrooms, such as *Lentinus edodes*, *Agaricus blazei*, *Ganoderma lucidum*, *Agaricus brasiliensis*, *Pleurotus florid*a, and *Lentinus squarrosulus*, as well as split gill mushrooms (Zhu et al., 2016). Mushroom β-glucans consist of non-starch polysaccharides characterized by a glucose polymer chain core featuring β-(1-3) linkages in the primary glucan chain, along with additional β-(1-6) branching points (Aramabašić Jovanović et al., 2021). The lengths of the core chains of β-glucan can vary, along with the diversity and intricacy of sidechain branching types. Mushroom β-glucans are not digested in the human gastrointestinal tract, as the enzymes secreted by intestinal brush border epithelial cells are unable to hydrolyze β-glycosidic bonds. Among other properties, they speed up the transit of bowel contents, increasing fecal bulk and frequency, with possible beneficial properties of protection against irritable bowel syndrome, diverticular diseases, and colon cancer (Gaullier et al., 2011). Polysaccharides may help alleviate diabetes through mechanisms involving gastrointestinal viscosity, inhibition of glucose absorption efficiency, and regulation of postprandial glycemia. Water-soluble dietary fibers and polysaccharides augment the viscosity of gastrointestinal contents, subsequently reducing the pace of gastric emptying (Guan et al., 2021). This delay in the digestion and absorption of carbohydrates contributes to their potential in managing diabetes. In addition, there are also indications that polysaccharides can bind and adsorb glucose, thus maintaining a low glucose concentration in the small intestine (Aramabašić Jovanović et al., 2021). Despite being fed a high-fat diet, the blood sugar levels in HFD+S240 groups that received high-dose polysaccharides from split gill were significantly more reduced than in HFD+S120 when compared with the HFD+DM group, Thus, it is pertinent to suggest that polysaccharides from split gill in high doses (240 mg) that contain a high concentration of β-glucan may be more effective in managing diabetes-related symptoms or complications in animals compared with low dosages. It was also able to prevent hyperglycemia by preventing insulin resistance in the rats fed a high-fat diet. The difference between the two dosages could also be related to the treatment duration. Depending on the study design, rats may receive the treatments for different lengths of time, and this could influence the observed effects. Several animal studies reported that decreased β-cell mass relative to elevated β-cell apoptosis, observed in the pancreas of diabetic animals, was effectively reversed after polysaccharide supplementation, and was accompanied by an elevated ratio of Bcl-2/Bax (Zhang et al., 2014). The prevalent characteristic observed in type 2 diabetes is directly associated with increased Bax expression and decreased levels of Bcl-2. Polysaccharides capable of stimulating cell proliferation have shown, upon administration to diabetic animals, histopathological evidence revealing increased β-cell mass, expanded pancreatic islets, and restoration (Zhu et al., 2014). Another protein-bound polysaccharide sourced from the fruit bodies of *G. lucidum* demonstrated comparable anti-diabetic potential by preventing β-cell apoptosis in streptozotocin (STZ)-induced diabetic rats (Zeng et al., 2017). This effect is associated with a significant increase in Bcl-2 expression and a decrease in Bax and caspase levels within pancreatic cells when compared to those observed in STZ-induced diabetic animals (Li et al., 2011).

Conclusion

A polysaccharide extract from split gill mushrooms, which is abundant in β-glucans, is effective in reversing the occurrence of hyperglycemia and insulin resistance in rats that were fed a high-fat diet in conjunction with a low dose of STZ. STZ induces diabetes type 2 by increasing the expression of insulin and GLUT2, which are recovered through the utilization of this extract. The β cells in the islets of Langerhans in the pancreas have the potential to be improved and restored. The relief or treatment of diabetes mellitus can be accomplished using this option for the treatment of many illnesses. The

creation of polysaccharide extracts from split gill mushrooms is also significant for the prevention of diabetes mellitus, the relief of symptoms associated with diabetes, or the treatment of diabetes as a complementary or alternative method of supplementation or as an herbal treatment. To investigate the possibility of its application in the treatment of diabetic patients in the future, additional research needs to be carried out.

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Data availability. Data will be made available upon request.

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