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Original Research Article

Evaluation of The Potency of Fermented Single-Bulb Garlic Cultured with *Lactobacillus plantarum* B1765 As An Antidiabetic In Type 2 Diabetic Rats

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ABSTRACT

The health-promoting components of single-bulb garlic are often enhanced by fermenting microorganisms during fermentation. Therefore, this research aimed to assess the antidiabetic effect of fermented single-bulb garlic (FSBG) using *Lactobacillus plantarum* B1765 on type 2 diabetes mellitus (T2DM) rats. The variables evaluated were peripheral blood glucose, venous blood glucose, and pancreatic *β*-cells. T2DM was induced in the experimental rats using a combination of a high-fat diet (HFD) and a low dose of streptozotocin (STZ). Antidiabetic effects of FSBG were compared with metformin, and the results showed that FSBG reduced peripheral blood glucose levels after 2–8 hours. In the longer term, 300 mg/kg of FSBG also significantly reduced venous blood glucose levels compared to metformin. The pancreatic *β*-cells were also significantly increased after 300 mg/kg FSBG oral doses. In conclusion, FSBG could be a potential source of antidiabetic constituents with good antidiabetic effects compared to conventional drugs such as metformin.

***Keywords*:** antidiabetic, pancreas, blood glucose, traditional fermented food, garlic, metformin, rats, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a chronic disease caused by metabolic disorders in pancreatic *β*-cells, leading to hyperglycemia. The two types of DM include type I diabetes mellitus (T1DM) and type II diabetes mellitus (T2DM). However, the prevalence of T2DM is significantly higher, reaching 85% among all DM, which is related to lifestyle and diet. Compared to T1DM, where hyperglycemia is caused by pancreatic dysfunction in producing insulin, T2DM pathophysiology includes insulin resistance in tissue and cells. T2DM causes severe complications, contributing to a significantly decreased quality of life and a high mortality rate when not managed appropriately. According to the International Diabetes Federation (IDF), in 2019, the number of T2DM patients in the 20–79 age group was 463 million, projected to reach 578 million in 2030. WHO data also shows that the mortality rate of T2DM will double in 2030, with the prevalence rate reaching 11.9% by 2045.[1] Several medical therapeutic procedures have been developed to control T2DM. However, the application of herbal medicine derivatives to manage T2DM has recently become increasingly popular due to the perceived rarity of side effects. Conventional medications for T2DM are often associated with severe side effects such as gastrointestinal disorders, impaired kidney function, decompensated heart and liver diseases, peripheral oedema, as well as acute hypoglycaemia. Therefore, new bioactive compounds isolated from plants have been proven more effective as antidiabetic agents for clinical purposes.

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Single-bulb garlic is a natural ingredient from cultivated plants, which has been extensively investigated for the potential to lower blood glucose levels in experimental animals.[2–5] The underlying mechanisms of antidiabetic properties have been explored in laboratory and animal research. According to Padiya (2013), long-term consumption of raw garlic was found to reduce insulin resistance in rats administered a fructose-rich diet.[6] The antioxidant properties of raw single-bulb garlic and isolated compounds also contribute to the positive effects on diabetes and associated complications. Additionally, garlic enhances glucose tolerance, stimulates glycogen synthesis[5], and restores insulin responsiveness.[7,8]

Despite the ongoing research on fresh single-bulb garlic, there is a limited exploration of fermented products. Compared to other plants, single-bulb garlic is rich in phenolic compounds[9], which are bound as glycosides and can be assessed through fermentation. Generally, fermentation breaks glycoside bonds using *β*-glucosidase produced by the microorganisms in the fermentation process to release the phenolic aglycon. This process is analogous to the deglycosylation of flavonoids in other natural sources.[10,11] Previous chemical analysis of garlic has identified various compounds, including alkaloids, terpenoids, flavonoids, steroids, phenols, anthraquinones, saponins, tannins, and glycosides.[12] Among these compounds, flavonoids are predominantly found as O-glycosides or C-glycosides in single-bulb garlic.[13] O-glycosylation diminishes the bioactivity of the compounds, decreasing antioxidant activity, antidiabetic effects, anti-inflammatory and antibacterial properties.[14] Therefore, O-glycosylation should be degraded to optimise the bioactivity of the flavonoid.

Previous *in vitro* research on fermented single-bulb garlic has shown more significant antioxidant activity and a stronger inhibitory effect on *α*-glucosidase enzyme compared to fresh garlic. These enhanced properties are associated with increased free phenolic compounds in fermented garlic, resulting from the activity of *β*-glucoside enzymes. Additionally, the results showed that fermented garlic could generate short-chain fatty acids (SCFAs), playing a significant role in antidiabetic mechanisms. SCFAs are formed through the breakdown of garlic's inulin by inulinase. The starter culture *Lactobacillus plantarum* B1765 could produce both *β*-glucosidase activity[15] and inulinase.[16] This phenomenon contributes to increased free phenolic compounds in fermented single-bulb garlic, which acts as an inhibitor of *α*-

glucosidase and produces SCFAs. The ability of *Lactobacillus plantarum* B1765 to produce *β*-glucosidase, which breaks glycoside bonds, is supported by the production of SCFAs through bacteria. Specifically, SCFAs decreased the pH of single-bulb garlic tissue, increasing the degradation of glycoside bonds.[17] Based on the background above, this research aimed to investigate the hypoglycaemic mechanism of administering FSBG to DM rats. The investigation evaluated the reduction in blood glucose levels and the significant impact on pancreatic *β*-cells.

Material and Methods

Animal preparation and type 2 diabetes mellitus inducement

Male Wistar rats (48), aged 8 weeks, weighing 150 and 170 grams, were selected as experimental subjects. Rats were procured from the Bioscience Laboratory at Brawijaya University in Indonesia and allowed to acclimatise for 2 weeks. Ethical approval was obtained from the Animal Care and Use Committee of Universitas Brawijaya (Approval ID: 068-KEP UB-2023; granted on June 14, 2023).

Rats were divided into 8 groups of 6 rats. The groups consist of the negative control (CN-healthy rats), positive control group (CP-diabetic induced but untreated), and treatment groups P1-P3 (Rats with induced T2DM and treated with 100, 200, and 300 mg/kg doses of orally administered FSBG, respectively. P4 comprised healthy rats treated with a 300 mg/kg dose of orally administered FSBG, and P5 included rats with induced T2DM and treated with a 9 mg/kg dose of orally administered metformin. Meanwhile, P6 consisted of healthy rats treated orally with a 9 mg/kg metformin dose.

Rats were fed a high-fat diet (HFD) of 40 grams of food for 22 days to induce T2DM. On the 23rd day, HFD was supplemented with a 20 mg/kg injection of streptozotocin (STZ) administered intraperitoneally, and this process was repeated for 7 days. Subsequently, rats were considered to have developed T2DM when the peripheral blood glucose levels exceeded 300 mg/dL.

Preparation of fermented single-bulb garlic paste.

The samples of single-bulb garlic (*Allium sativum* L.) were collected from a farmer in Pasuruan, East Java who collected their products to Koperasi Kabupaten Pasuruan (Area Sawah/Kebun, Raci, Kecamatan Bangil, Pasuruan, Jawa Timur 67153), in May 2023. Then, the single-bulb garlic was washed and peeled. Approximately 250 grams was placed into a sterile glass jar containing 250 mL of a 5% salt solution. This was followed by adding 10% (v/w) *Lactobacillus plantarum* B1765 starter culture and fermented at 37.0°C for 9 days until FSBG was formed. Subsequently, FSBG was converted into a freeze-dry preparation before adding sterile distilled water with a weight ratio of 1:1 to obtain a paste form.[18] After establishing DM (increase in peripheral blood glucose of more than 300 mg/Dl), FSBG paste was administered orally thrice a day at 8-hour intervals for 14 consecutive days, as previously reported.[19] When the paste was excessively thick, dilution was performed 10 times in sterile distilled water before administration.

Evaluation of peripheral blood glucose level after the single-dose administration of FSBG

The peripheral blood glucose level was measured using Gluco DR Biosensor (Allmedicus, South Korea) sequentially after a single administration of FSBG paste of 100 mg/kg, 200 mg/kg, and 300 mg/kg at intervals of 2 hours, 4 hours, 6 hours, and 8 hours, respectively.[20]

Evaluation of venous blood glucose level

The venous blood specimens were collected from the right retroorbital vein. The glucose levels of the blood and urine specimens were measured using an ELISA kit (Cat. Number SL1110Ra, SUNLONG Biotech, China) and an ELISA reader (SPECTROstar Nano, BMG Labtech, Germany), respectively. The specimens were collected in phases, which included a day before HFD was administered, the 23rd day before the injection of STZ, the last day of T2DM induction, the last day of administering fermented single-bulb garlic paste, and the 2nd week after the cessation of the fermented single-bulb garlic paste administration.[21]

Pancreatic Tissue Evaluation

After the last venous blood collection, rats were sacrificed for organ collection. To assess the morphology of pancreatic tissue, specifically *β*-cells, the pancreas was processed into a paraffin block preparation, sliced into 4 micrometres thick, and stained with haematoxylin-eosin. The tissue was observed at 400 times magnification using an Olympus BX53 Microscope equipped with CellSens software (BX53, Japan).[22]

Statistical Analysis

Data was analysed using ANOVA and Tukey-Post Hoc test for significant differences.

Results and Discussion

The study evaluated the effect of single-dose administration of FSBG on peripheral blood glucose levels. As presented in Table 1, peripheral blood glucose levels showed a significant change after a single-dose administration of FSBG. Linear regression analysing the correlation between increased doses of FSBG and the timing showed a significant negative association with $p< 0.001$ and beta-coefficient -0.787 (comprehensively). Figure 1 shows a significant reduction of peripheral blood glucose 2 hours after administration of FSBP, followed by a less prominent decrease during subsequent observation periods.

Phenolic compounds produced during FSBG fermentation are known to lower blood glucose levels by increasing the inhibitory activity of the *α*-glucosidase enzyme. According to previous research, FSBG was proven to have higher total phenolics *in vitro* than fresh garlic. The total phenolics correlated with stronger inhibitory efficacy against *α*-glucosidase enzyme activity than acarbose as a control.[23] According to Lee (2016), solid garlic fermented with Lactic Acid Bacteria has stronger *α*-glucosidase enzyme inhibitory activity compared to acarbose.[24] Fujita (2017) also stated a significant increase in *α*-amylase and *α*-glucosidase inhibition in fermented products.[25] Another mechanism in reducing blood glucose levels is related to the formation of short-chain fatty acids (SCFAs) in FSBG products. Specifically, SCFAs are formed from the inulin metabolism in single garlic by the inulinase activity produced through *L. plantarum* B1765, serving as a starter culture in FSBG.[16]

In previous *in vitro* research, FSBG had been proven to produce SCFAs such as acetic, propionic, and butyric acids. These SCFAs were produced through the hydrolysis of the soluble fibre inulin by inulinase of *L. plantarum* B1765. Subsequently, soluble fibre was fermented by gut microbiota to produce SCFAs, which entered the blood circulation. This phenomenon affects glucose storage in the liver and muscles, reducing food consumption by Acetate (C2), which reduces appetite.[26]

Table 1: Peripheral Blood Glucose Levels after the Administration of Single-Dose FSBG

Dose of FSBG	Levels of Peripheral Blood Glucose (mg/dL)				
	Before FSBG administration	**2 hours after FSBG administration**	**4 hours after FSBG administration**	**6 hours after FSBG administration**	**8 hours after FSBG administration**
100 mg/kg	540 mg/dL	471 mg/dL	399 mg/dL	386 mg/dL	392 mg/dL
200 mg/kg	545 mg/dL	473 mg/dL	454 mg/dL	434 mg/dL	408 mg/dL
300 mg/kg	560 mg/dL	424 mg/dL	407 mg/dL	405 mg/dL	378 mg/dL

Zhao (2018) found that SCFAs-producing microorganisms thrive in T2DM patients with the intervention of a high-fibre diet. This correlated with improved blood glucose regulation, elevated glucagon-like peptide-1 (GLP-1) levels, and decreased acetylated haemoglobin levels.[27] SCFAs are known to elevate GLP-1 and peptide-tyrosine-tyrosine (PYY) secretion. GLP-1 enhances the secretion of glucose-dependent insulin, while PYY suppresses appetite, inhibits gastrointestinal movement, and improves the survival and function of pancreatic β-cells, offering benefits for individuals with T2DM [(28)]. Also, the results of the effect of the routine administration of FSBG on venous blood glucose levels are shown in Table 2. The result revealed a significant change in venous blood glucose levels after the routine administration of FSBG, specifically between CP and P3. The decrease in venous blood glucose levels in T2DM rats after receiving oral therapy (both FSBG and metformin) for 2 weeks is shown in Figure 2 (blue bar diagram). Based on the results, significant changes were observed in venous blood glucose levels 14 days after the cessation of oral therapy. Fermentation is widely recognised for altering various organic components, causing an increase in the bioactivity of products.[29] In previous research, FSBG showed significant potential to increase antioxidant activity *in vitro*[18], and free phenolic compounds correlate with inhibition of α-glucosidase enzyme activity (unpublished data). Meanwhile, in this research, the effect of FSBG administration on T2DM rats induced by HFD for 22 days, followed by STZ at a dose of 20 mg/kg for 7 days, was observed. The results showed a significant increase in peripheral blood glucose levels, reinforcing the antidiabetic effects of FSBG.

This study showed that feeding diabetic rats 300 mg/kg BW FSBG juice for 14 days could return hyperglycemia levels of T2DM rats to normal. Despite a slight increase in blood glucose levels after 14 days without eating, rats remained in the usual range, indicating the significant effects of FSBG compared to metformin. Several investigations showed that fermentation products reduced blood glucose levels. For example, *in vivo* research on T2DM rats given a decoction extract of fermented purple Jerusalem artichoke showed reductions in blood glucose levels and α-glucosidase activity while increasing insulin levels.[30]

Table 2: The Venous Blood Glucose Levels after Routine Administration of FSBG (mg/dL)

Group according to dose	0th Day (a day before HFD was administered)	23rd day (before STZ was injected)	29th day (last day of T2DM inducement)	44th day (last day of FSBG oral administration)	59th day (2 weeks after the FSBG has been ceased)
CN	127.5	127	129	117.3	145.6
CP	117.4	221.4	580.7	531.5	509.5
P1	120.8	217.9	510.1	113.1	139.3
P2	134.6	216.6	545.6	124.9	127.2
P3	124	205.5	559.2	96.4	157.1
P4	148.3	127.9	122.6	102.6	155.2
P5	116.3	229.7	529.3	135.7	162.2
P6	131.3	131.4	124.9	122.5	149.6

(CN: healthy rats; CP: T2DM-induced rats without treatment; P1: T2DM-induced rats treated with a 100 mg/kg FSBG; P2: T2DM-induced rats treated with a 200 mg/kg FSBG; P3: T2DM-induced rats treated with a 300 mg/kg FSBG; P4: healthy rats treated with a 300 mg/kg FSBG; P5: T2DM induced rats treated with a 9 mg/kg metformin; and P6: healthy rats treated with a 9 mg/kg metformin)

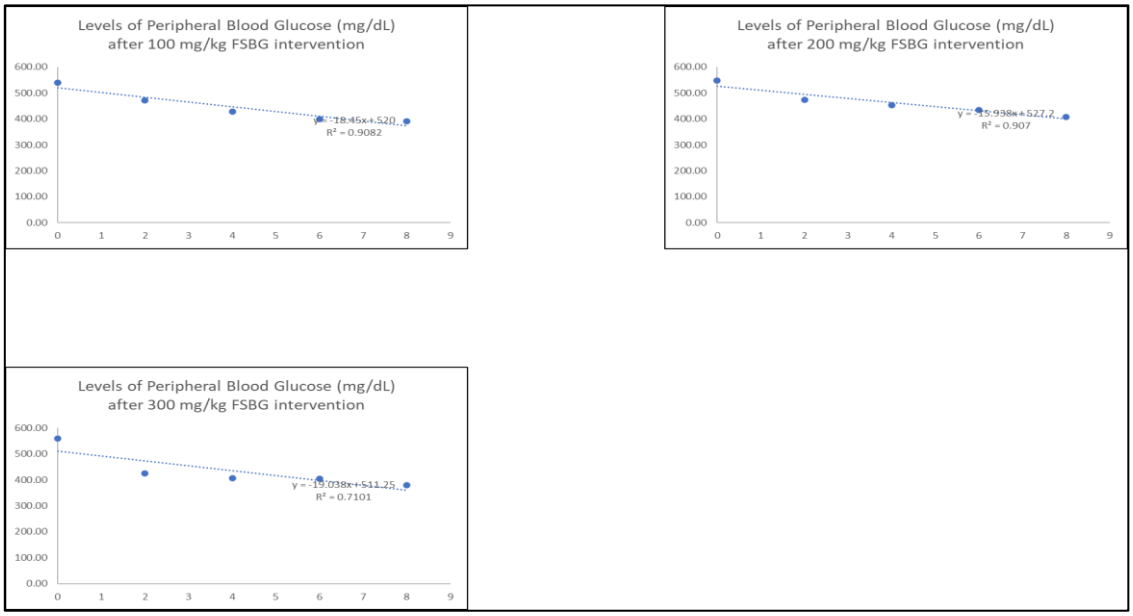

Figure 1: The Reduction of the Peripheral Blood Glucose Levels (mg/dL). (A: Chart of the blood glucose levels for two-hour intervals following 100 mg/kg FSBG, R^2=0.9082 means 90.8% reduction of blood glucose levels happened due to intervention. B: Chart of the blood glucose levels for two-hour intervals following 200 mg/kg FSBG, R^2=0.907 means 90.7% reduction of blood glucose levels happened due to intervention. C: Chart of the blood glucose levels for two-hour intervals following 300 mg/kg FSBG, R^2=0.7101 means 71.0% reduction of blood glucose levels happened due to intervention.)

Previous research on fermented rice bran and soybeans derived from *Bacillus* subsites showed antidiabetic effects, as indicated by reduced blood glucose and HbA1C levels in T2DM-induced rats.[31] Fermented *Morinda citrofila* L. is also known to lower blood glucose and HbA1C levels while improving insulin sensitivity.[32] Supplementation of fermented skim milk made by Probiotic *L. fermentum* showed reduced blood glucose in alloxan-induced DM rats compared to controls.[33] In 2022, another investigation performed in India showed that compared to tomato and carrot, garlic or a combination with other extracts was the most potent antidiabetic herbal medicine when tested in an alloxan-induced T2DM animal model.[34] Similarly, the effect of the routine administration of FSBG on the number and the morphology of pancreatic *β*-cells is shown in Figure 3. Induction of T2DM using HFD and STZ injection significantly reduced the number of *β*-cells in the pancreas, as evidenced by the p-value between CN and CP of 0.009. When T2DM-induced rats were administered 300 mg/kg of FSBG for 2 weeks (P3), the number of pancreatic *β*-cells increased and significantly differed from the CP group, based on the p-value between CN and CP of 0.030. Compared to metformin therapy in rats of the P5 group, FSBG therapy produced better results than metformin, with a p-value of 0.790 between CP and P5.

The differences in pancreatic *β*-cell morphology between CN, CP, and P3 groups are presented in Figure 4. The results showed that in CN, *β*-cells appeared intact with preserved nuclei (blue arrows), while CP had damaged and dysmorphic nuclei. Meanwhile, rats induced diabetes and administered FSBG (P3) showed pancreatic *β*-cells that had returned to intact state.

This study proved the potential of FSBG administration to increase the number of *β*-cells in T2DM-induced rats. The results showed that T2DM caused necrosis and decreased the number of *β*-cells in the pancreas after rats were exposed to HFD and STZ. Meanwhile, the administration of FSBG increased the number of *β*-cells similar to normal rats superior to those treated with metformin. The morphology of pancreatic *β*-cells was improved after 300 mg/kg of FSBG was administered. In 2023, previous research conducted in Mexico showed that *Lactobacillus casei* produced fermented sodium alginate with high levels of antioxidants. These significant characteristics enhanced the condition of pancreatic *β*-cells, resulting in increased cells after fermented sodium alginate therapy.[35] A scientific review published in 2020 also showed higher levels of antioxidants, specifically phenolic compounds, procured from fermented legumes, vegetables, and fruits. These identified phenolic compounds included lignin, proanthocyanin, catechin, kaempferol, flavonoids, and quercetin.[36]

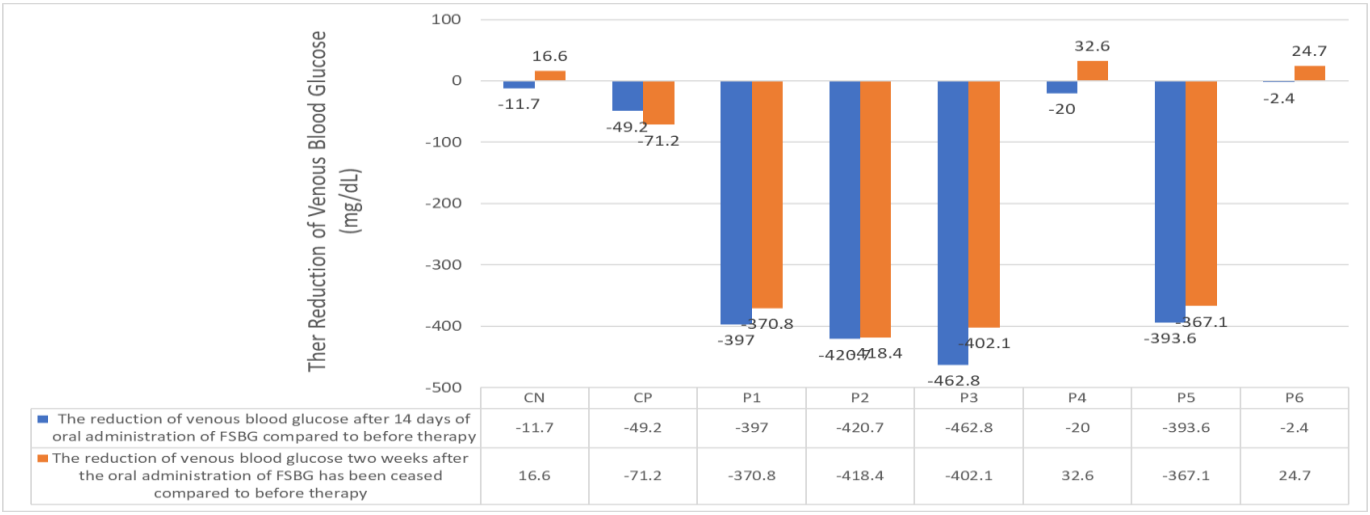

Figure 2. The Reduction of the Venous Blood Glucose Levels (CN: healthy rats; CP: T2DM induced rats without treatment; P1: T2DM induced rats treated with a 100 mg/kg FSBG; P2: T2DM induced rats treated with a 200 mg/kg FSBG; P3: T2DM induced rats treated with a 300 mg/kg FSBG; P4: healthy rats treated with a 300 mg/kg FSBG; P5: T2DM induced rats treated with a 9 mg/kg metformin; and P6: healthy rats treated with a 9 mg/kg metformin)

Conclusion

This research showed that FSBG derived from *Lactobacillus plantarum* B1765 had potential antidiabetic properties. With the optimal 300 mg/kg dose, administering FSBG could return the blood glucose levels to normal. The results showed that the reduction in blood glucose levels after FSBG administration was superior to conventional metformin therapy in the short and long term. Additionally, FSBG administration increased the number of *β*-cells in the pancreas more effectively compared to metformin therapy. Based on these potential antidiabetic effects, proper formulation and further clinical trials on using FSGB in managing Type II diabetes mellitus should be considered for comprehensive results.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

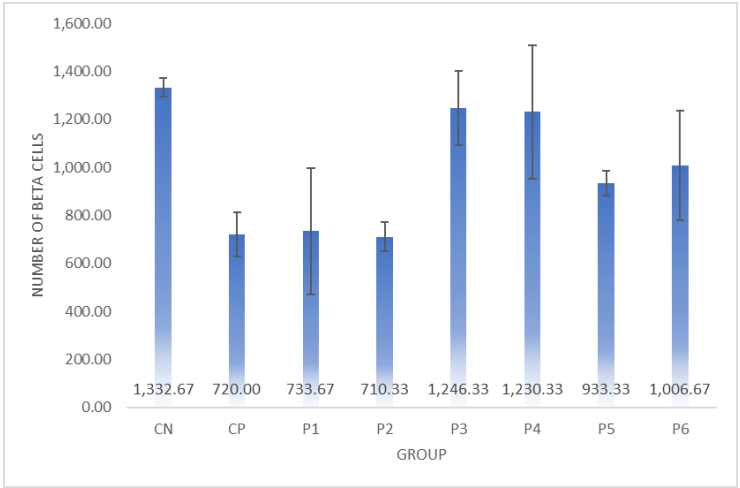

Figure 3: The Number of Pancreatic β-Cells after FSBG Therapy (CN: healthy rats; CP: T2DM-induced rats without treatment; P1: T2DM-induced rats treated with a 100 mg/kg

FSBG; P2: T2DM-induced rats treated with a 200 mg/kg FSBG; P3: T2DM induced rats treated with a 300 mg/kg FSBG; P4: healthy rats treated with a 300 mg/kg FSBG; P5: T2DM induced rats treated with a 9 mg/kg metformin; and P6: healthy rats treated with a 9 mg/kg metformin)

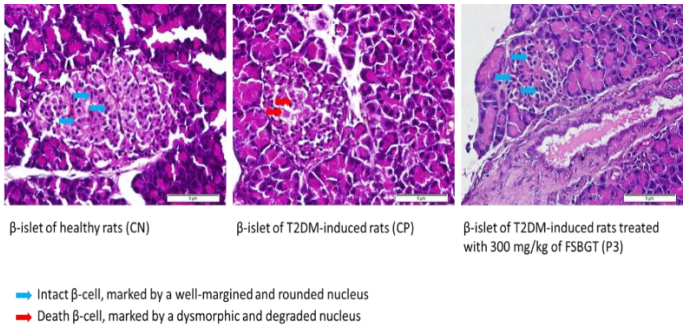

Figure 4: The Morphology of Pancreatic β-Cells after FSBG Therapy

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