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The Characterization of Fructose-Based High-Fat Diet and Low-Dose Streptozotocin in A Type 2 Diabetes Mellitus Rat Model

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ABSTRACT

Information on the biochemical profiles of new antidiabetic agents from animal models is essential for preclinical trials. This study optimizes and characterizes a high-fat, high-fructose diet (HFFD) and low-dose streptozotocin (STZ)-induced type 2 diabetes mellitus (type 2 DM). Wistar rats were fed an HFFD (2 ml of egg yolk, 2 ml of lard, and fructose at 3.6 g/kg BW) for 2, 4, and 6 weeks (p.o), followed by STZ (35 mg/kg body weight [BW], i.p) to measure the biochemical parameters. Non-induction HFFD and STZ were used as a normal control group. HFFD for 2, 4, and 6 weeks before STZ injection and low-dose STZ-induced rats demonstrated an elevation in the body weight, fasting blood glucose, triglyceride, triglyceride/ glucose index, necrosis score, and insulin-negative cells. Moreover, this induction also reduced the number of insulin-positive cells and the percentage of insulin-positive cells. Among all treatments, HFFD for 2 weeks before STZ injection and low-dose STZ succeeded the type 2 DM rat model and showed stability for 3 weeks after STZ induction. These findings imply that feeding Wistar rat HFFD for 2 weeks, followed by a single dose of STZ at 35 mg/kg BW is a reliable and stable diabetic rat model closely resembling the biochemical characteristics of type 2 DM.

Keywords: Fructose, high-fat diet, streptozotocin, diabetic model, Wistar rats

INTRODUCTION

Diabetes mellitus (DM) is a global health problem, rapidly increasing metabolic disease cases worldwide (Wu et al., 2021). In 2019, disease cases were estimated to be 463 million, and were predicted to increase to 700 million by 2045 (Saeedi et al., 2019). Over 90% of patients diagnosed with type 2 DM had β-cell dysfunction and abnormal carbohydrate, lipid, and protein metabolism (Li et al., 2019; Wu et al., 2021). This phenomenon precedes β-cell dysfunction, impairs abnormal insulin secretion, and causes an imbalance of glucose levels (Li et al., 2019). Hyperglycemic conditions have defective hyperinsulinemia, that promotes insulin resistance through an insulin-dependent pathway (Patel et al., 2016). Insulin resistance can occur in several organs and tissues, including the liver, the heart, adipose tissue, and skeletal muscle (Wu et al., 2021). Long-term exposure to these conditions in the organs and tissues leads to micro- and macrovascular complications, including nephropathy, liver damage, retinopathy, neuropathy, and others (Arifah et al., 2022a; Sadeghabadi et al., 2022).

In drug discovery efforts, pharmacological studies play an important role in evaluating efficacy, potency, mechanism of action, and target of action. These studies can be carried out in vitro and in vivo (Nugroho et al., 2011; Mutmainah et al., 2014; Nugroho et al., 2016; Arifah et al., 2022b). Animal models help investigate the effects and mechanism of action of agents as antidiabetics, while the models can provide invaluable

information on the pathophysiological conditions of type 2 DM (Barrière et al., 2018; Guo et al., 2018). Type 2 DM is a complicated disease involving hereditary and environmental variables; subsequently, the animal models closely mimic the natural progression of type 2 DM from insulin resistance to β-cell damage (Guo et al., 2018; Prasad & Groop, 2015). Therefore, the translational value of the animal model can be improved by decreasing the gaps between preclinical and clinical levels (Barrière et al., 2018).

Many animal models represent type 2 DM, such as streptozotocin (STZ), high-fat, high-fructose diet (HFFD), or combinations. High doses of STZ severely defect insulin secretion and are related to type 1 DM; low-dose STZ causes mild impairment in insulin secretion and is more closely related to the later stages of type 2 DM (Gheibi et al., 2017). On the other hand, HFFD diet refers only to prediabetes and never develops β-cell destruction as observed in advanced stages of type 2 DM in humans (Barrière et al., 2018). Low-dose STZ induction does not appear to cause insulin resistance, so that, HFFD diet induction and followed by low-dose STZ can be applied to develop type 2 DM (Barrière et al., 2018; Gheibi et al., 2017).

Recently, many animal models used low-dose STZ and a high-fat diet (HFD), sucrose, or fructose to develop type 2 DM (Barrière et al., 2018; Skovsø, 2014). A previous study conducted type 2 DM using syrup solution (8.8% glucose and 5.2% fructose) for 6 weeks, and STZ (45 and 65 mg/kg BW) showed β-cell dysfunction and insulin resistance condition in rats (Artemisa & Adolfo, 2021). High-fat diet (24.8% w/w fat, 54.6% w/w carbohydrate, and 12.8% w/w protein) for 4 weeks, and STZ injection (30, 40, and 50 mg/kg BW, i.p) was also conducted to make type 2 DM rat models (Wickramasinghe et al., 2022). Meanwhile, these type 2 DM rat models did not use a combination of fat and fructose due to similar unhealthy diets in humans (Barrière et al., 2018). HFFD (46.6 wt% fructose and 25.7% lard) for 56 weeks and multiple low-dose STZ (25 mg/kg BW, i.p., three times in early treatment every 6 weeks) to develop type 2 DM with complications (Barrière et al., 2018). However, multiple injections have a probability of more discomfort in animals and may influence the reproducibility of the results due to the risk of injection errors (Wickramasinghe et al., 2022). Furthermore, appropriate, low-cost, quick, and easy replicated are essential issues in developing type 2 DM anima l models. Hence, this study provided type 2 DM animal models that encountered main diet stressors in humans (HFFD) with low-dose STZ.

MATERIALS AND METHODS

Chemicals

Streptozotocin (Cayman), fructose (Merck), egg yolk, lard, aquadest, citrate buffer 0.1 M (pH 4.5), glucose kit (Dyasis), insulin kit (Finetest), triglyceride kit (Dyasis), creatinine kit (Dyasis), alanine transaminase (ALT) kit (Dyasis), formalin 10%, chemicals for histology, and chemicals for immunohistochemistry (IHC) (UltraTek HRP Anti-Polyvalent [DAB]).

Animals

Male Wistar rats (8 – 10 weeks old) were used in the experiments conducted in the Faculty of Pharmacy, Universitas Gadjah Mada (UGM). The research procedure was authorized by the Institutional Animal Ethical Committee, the Integrated Research and Testing Laboratory (LPPT), UGM with the number 00018/04/LPPT/V/2021. Rats were maintained in polypropylene cages at a temperature of 22^{o}C ± 2^{o}C with a relative humidity of 55% ± 10% and a 12 h automated light/dark cycle. They were provided standard laboratory animal feed and water *ad libitum*.

Experimental design

Twenty-four rats were divided into four groups (n = 6) (Figure 1). Group I was the control normal group. Groups II, III, and IV were induced by a high-fat diet (HFD) (2 ml of egg yolk and 2 ml of lard, p.o) and fructose (3.6 g/kg body weight [BW], p.o) throughout the period. Also, STZ (35 mg/kg BW, i.p) was injected on days 14, 28, and 42 for groups II, III, and IV, respectively. Fructose was dissolved using aquadest. STZ was freshly made before injection and dissolved in ice-cold 0.1 M citrate buffer, pH 4.5.

The BW of rats in all groups was observed once per week; fasting blood glucose (FBG), triglyceride, and fasting triglyceride/ glucose index (TyG index) were measured at all points (Figure 1). Several parameters, including fasting serum insulin (FINS), homeostasis model assessment-insulin resistance (HOMA-IR), creatinine, and ALT were measured on days 0, 7 days after STZ injection, and terminal days of the experiment period. Rats were fasted overnight before the blood sampling through the orbital sinus.

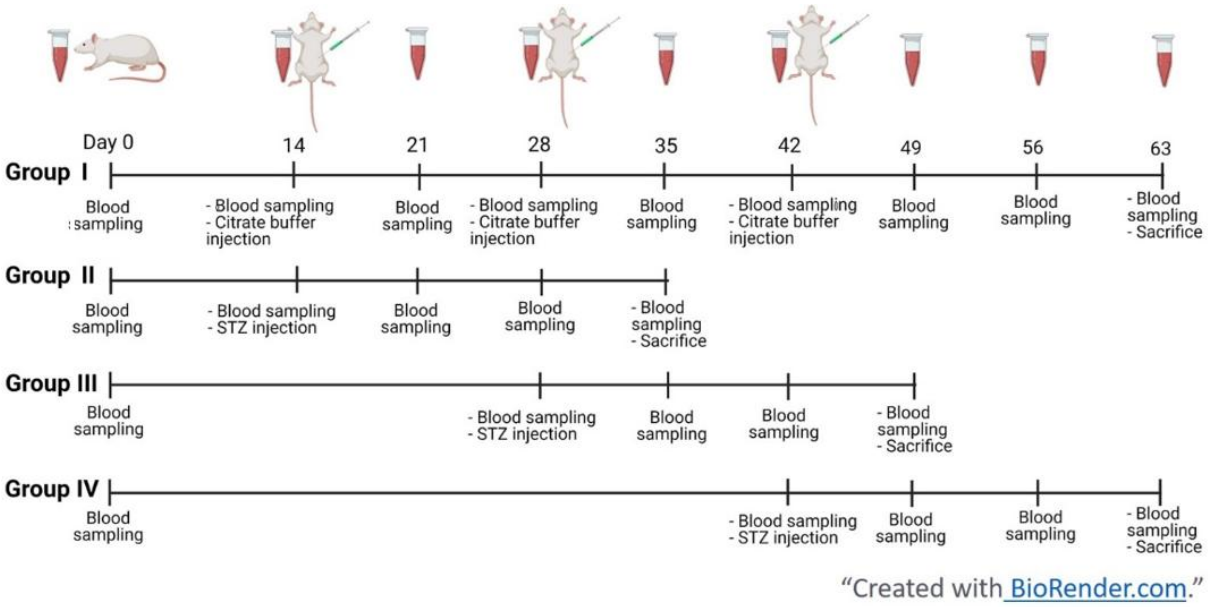

Figure 1. The research design of the diabetic rat model

Blood samples were allowed to stand at room temperature for approximately 30 minutes then centrifuged at 5000 rpm for 10 minutes to obtain sera for measuring the biochemical parameters. After the experiment, all rats were sacrificed, and several organs were collected for further analysis, involving organ index and histology (pancreas, kidney, liver, heart) and IHC (pancreas).

Parameters measured

Determination of BW, blood biochemical, and insulin resistance predictors

The BW was measured using Ohauss mechanical balance. The biochemical parameters assessed included FBG, fasting triglyceride, FINS, creatinine, and ALT. Thus, insulin resistance predictors include the TyG index and HOMA-IR. The FBG and fasting triglyceride parameters were measured using Microlab 300 following the procedures described by DiaSys, Germany. FINS in the serum was measured using the enzyme-linked immune-sorbent assay (ELISA) method following the procedures described by FineTest, China. Creatinine (Jaffe method) and ALT were measured using Microlab 300 following the procedures described by DiaSys, Germany. Creatinine (Jaffe method) and ALT were measured using Microlab 300 following the procedures described by DiaSys, Germany.

Organ index and its histological examination

The organ index of four organs (n = 6) with the following formula: (organ weight/ BW) x 100 (Aamir et al., 2022). For the kidney, we used the average for this assessment. Three organs from each group were used for histological analysis with hematoxylin-eosin (HE) stained and examined for necrosis changes (Badole et al., 2015; Mangunsudirdjo, 1990; Motshakeri et al., 2014). The microscopic images were examined using a light microscope (Olympus), and the photomicrographs were taken at 40 x magnification (Antony et al., 2017). Necrosis degree is scored as follows: normal (0% necrosis) (-), mild (1%-25% necrosis) (+), moderate (26%-50% necrosis) (++), and severe (>50% necrosis) (+++) (Gillespie et al., 2011).

Pancreatic immunohistochemistry

Animals (n = 3) from each group were sacrificed for IHC analysis. The thick pancreas (3 mm) was dried, deparaffinized, rehydrated, washed under running water, pretreated with Tris EDTA pH 9.0 at 95°C, cooled, and washed with PBS pH 7.4. The samples incubated with peroxidase block, superblock, primary antibody, anti-polyvalent UltraTek, UltraTek HRP, and DAB solution, where each incubation process was washed first with PBS pH 7.4. When the samples were incubated with DAB solution, they were washed under running water, soaked with bluing reagent, and washed under running alcohol. The process continued with dehydration, clearing, mounting, and covering with a glass coverslip (Medipath Science Indonesia, 2022). The pancreatic sections were analyzed under a light

microscope (Olympus) with a magnification of 400x (Subash-Babu et al., 2009). The sections examined the percentage of insulin-positive cells in Islet cells (Langerhans island) by selecting five islet cells from five field views using ImageRaster software (Abunasef et al., 2014; Chandrasekaran et al., 2018). The percentage of insulin-positive cells with the following formula: (insulin-positive cells/ (insulin-positive cells + insulin-negative cells)) x 100 (Ahmed et al., 1998).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM) and analyzed using SPSS version 23 at $p < 0.05$ as a significant level. Data were analyzed using the analysis of variance test followed by a post-hoc Tukey test for multiple comparisons of normal and homogenous data (Hamadi et al., 2012). Furthermore, abnormal and/ or non-homogenous data were analyzed using the Mann-Whitney test.

RESULTS AND DISCUSSIONS

Effects on body weight, blood biochemical, and insulin resistance predictors

The effects of HFFD induction and low-dose STZ on BW, FBG, fasting triglyceride, FINS, creatinine, ALT, TyG index, and HOMA-IR are shown in Figure 2. The BW (Figure 2A) showed no significant difference when all treatment groups were compared with group I ($p < 0.05$). Groups II, III, and IV on days 28, 14, and 21, began the elevation until the final day compared with day 0. Moreover, Groups II, III, and IV increased by 1.39, 1.33, and 1.57 times on the final day when compared with day 0. FBG data should be measured on day 7 after STZ injection, and 126 mg/dl indicated diabetes (Centers for Disease Control and Prevention, 2021; Guo et al., 2018). The FBG levels of all treatment groups increased significantly compared with groups I and more than 126 mg/dl (Figure 2B). Group II showed the highest FBG elevation compared with group I, i.e., 2.81. The FBG level of all groups was also compared between sampling points, and all treatment groups showed a significant increase compared with day 0. Group II showed the highest FBG level after 7 days of STZ injection compared with day 0, i.e., 6.58. However, this study showed that group I also increased significantly compared with day 0.

Regarding the triglyceride levels of all treatment groups in the borderline-high range (150 – 199 mg/dl) after STZ injection (Zhao et al., 2019); only group II remained stable until day 21 after STZ injection (Figure 2C). Group II on days 7 and 21 after STZ injection and group IV on day 21 after STZ injection revealed a significant elevation compared with group I. Furthermore, = on day 7 after STZ injection, group II revealed the highest triglyceride level when compared with group I, i.e., 2.13 times. The triglyceride levels of all groups were also compared between sampling points, and all treatment groups showed a significant increase compared with day 0. On day 7 after STZ injection, group II revealed the highest triglyceride levels compared with group I on day 0, i.e., 3.15. However, several groups had decreased triglyceride levels after 7days of STZ injection.

For FINS data (Figure 2D), groups II, III, and IV had changes in the FINS level However, no group had a significant increase in insulin level compared with group I (normal treatment) and day 0. Thus, the effects of the HFFD induction and low-dose STZ on creatinine and ALT levels (Figures 2E and 2F). Each sampling point and group were analyzed statistically by comparing group I and day 0, respectively. However, all treatment groups showed no difference statistically in creatinine and ALT levels.

TyG index analysis revealed that groups II and IV on days 7, 21, and 28 after STZ injection showed significant elevation compared with group I (Figure 2G). Group II on day 7 after STZ injection showed the highest TyG index compared with group I, i.e., 1.18. The TyG index of all groups was also compared between sampling points, and all treatment groups demonstrated a significant increase compared with day 0. Group II, on day 7 after STZ injection, revealed the highest TyG index compared with day 0, i.e., 1.36. However, group I also increased significantly compared with day 0.

HOMA-IR was also measured (Figure 2H), and showed that group II on day 7 and group III on days 7 and 21 after STZ injection had significant elevation compared with group I. Group II on day 7 after STZ injection demonstrated the highest HOMA-IR compared with group I, i.e., 6.47. The HOMA-IR of all groups was also compared between sampling points, and all treatment groups showed a significant increase compared with day 0. Group II on day 7 after STZ injection, showed the highest HOMA-IR compared with day 0, i.e., 8.89 times. However, group I also increased significantly compared with day 0.

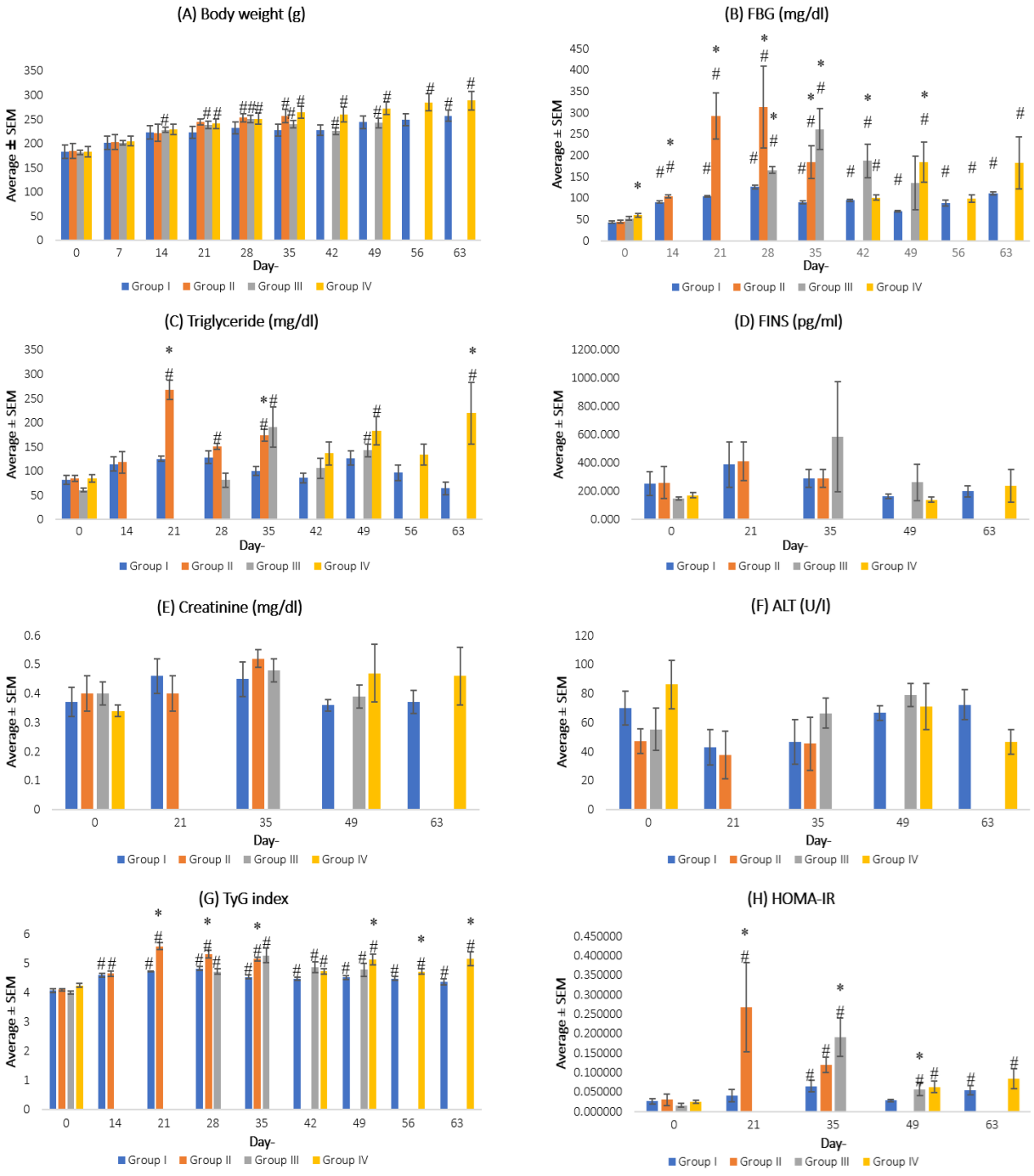

Figure 2. Effects on body weight, fasting blood glucose (FBG), triglyceride, fasting insulin (FINS), creatinine, alanine transaminase (ALT), TyG index, and homeostasis model assessment-insulin resistance (HOMA-IR). Values are mean ± SEM, n = 4 – 6 animals per group. All values are $p < 0.05$, which was considered statistically significant. (#) the significant difference with day 0 and (*) significant difference with group I.

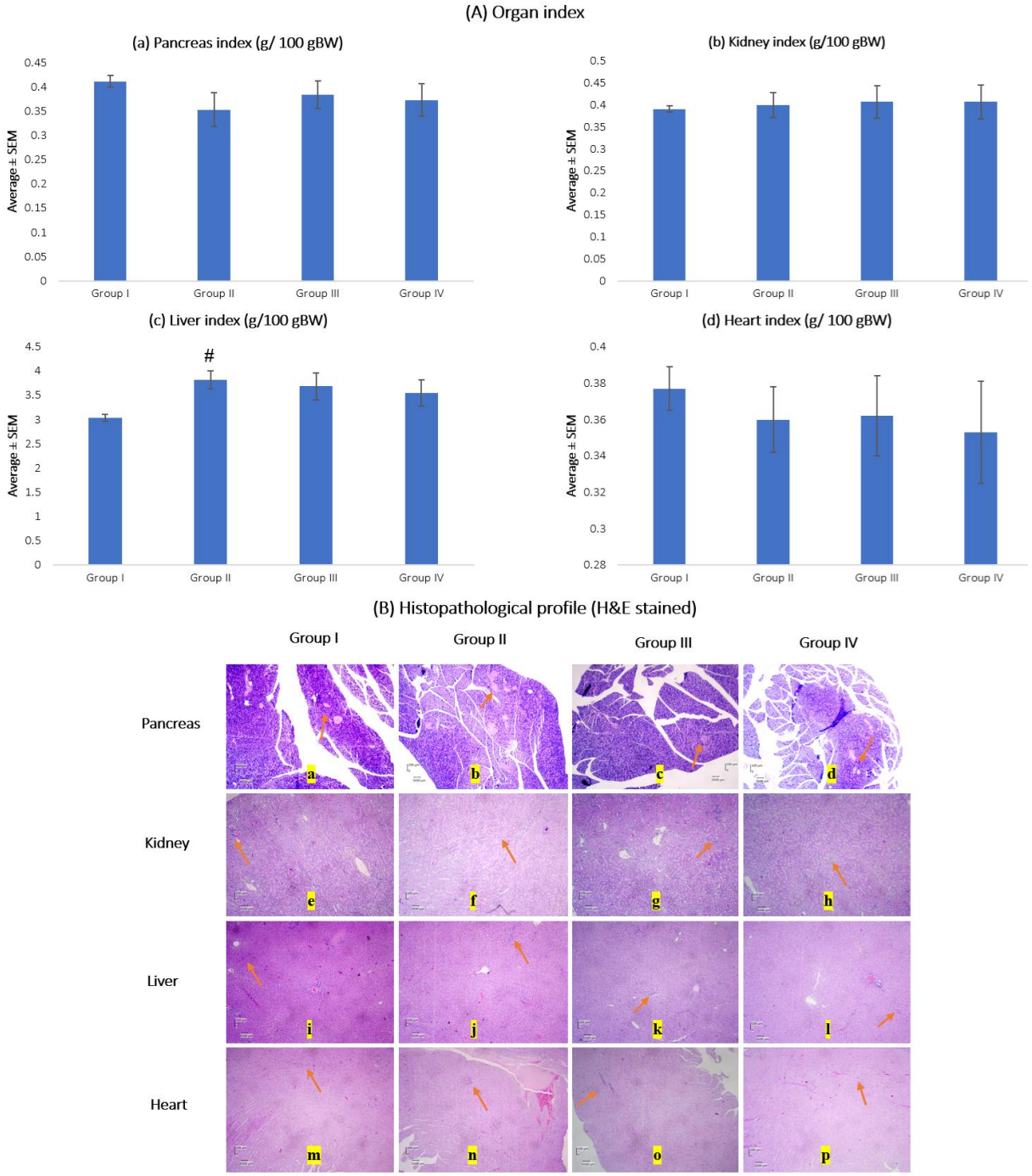

Figure 3. (A) Effects on organ index. (a) pancreas index, (b) kidney index, (c) liver index, and (d) heart index. Values are mean ± SEM, n = 4 – 6 animals per group. All values are $p < 0.05$, which was considered statistically significant. (#) the significant difference with day 0 and (*) significant difference with the group I. (B) Photomicrographs of pancreas, kidney, liver, and heart sections stained by H&E (40 x). (a) pancreas of group I, (b) pancreas of group II, (c) pancreas of group III, (d) pancreas of group IV, (e) kidney of group I, (f) kidney of group II, (g) kidney of group III, (h) kidney of group IV, (i) liver of group I, (j) liver of group II, (k) liver of group III, (l) liver of group IV, (m) heart of group I, (n) heart of group II, (o) heart of group III, (p) heart of group IV.

Table I. Effects on necrosis score, n = 3 organs per group

	Pancreas				Kidney			
	Normal	**Mild**	**Moderate**	**Severe**	**Normal**	**Mild**	**Moderate**	**Severe**
Group I	3	0	0	0	1	2	0	0
Group II	2	1	0	0	0	3	0	0
Group III	2	1	0	0	1	2	0	0
Group IV	1	2	0	0	0	3	0	0
	Liver				**Heart**			
	Normal	**Mild**	**Moderate**	**Severe**	**Normal**	**Mild**	**Moderate**	**Severe**
Group I	3	0	0	0	2	1	0	0
Group II	1	2	0	0	2	1	0	0
Group III	1	2	0	0	2	1	0	0
Group IV	1	2	0	0	2	1	0	0

Effects on organ index and its histological examination

The effects of the induction of HFFD and low-dose STZ on the organ index (pancreas, kidney, liver, and heart) (Figure 3A). Each sampling point and the group were analyzed statistically by comparing group I (normal treatment) and day 0, respectively. This analysis showed that only group II significantly increased liver index.

The effects of the induction of HFFD and low-dose STZ on the necrosis score of several organs, such as the pancreas, kidney, liver, and heart (Table I and Figure 3B). Each group was analyzed descriptively by the number of necrosis scores and microscopic architecture of several organs. In this study, group I, normal treatment, showed normal architecture in the pancreas and liver but normal and mild necrosis in the kidney and heart. All treatment groups showed increased necrosis in the pancreas, kidney, liver, and heart (Table I).

Effects on pancreatic immunohistochemistry

The effects of HFFD induction and low-dose STZ on pancreatic immunohistochemistry (Table II, Figure 4). Each group was analyzed statistically by the number of insulin-positive cells, number of insulin-negative cells, percentage of insulin-positive cells, and microscopic architecture. Administration of HFFD and low-dosage STZ showed a reduction in the number and percentage of insulin-positive cells and an increase in the number of insulin-negative cells (Table II). Group III exhibited a significant reduction in the percentage of insulin-positive cells. Also, group IV significantly reduced the total number of insulin-positive cells from five islets and the average number of insulin-positive cells per islet.

The photomicrographs of pancreatic islets observed insulin-positive expression marked as dark brown granules in the cytoplasm of β cells (Abunasef et al., 2014; Dhanavathy, 2015). Insulin-positive and insulin-negative cells are characterized by the presence or absence of a nucleus. Group I, as the normal control, showed strong immuno-reactivity for anti-insulin antibodies, marked by deep brown granules (Figure 4a). This phenomenon indicates the presence of insulin in the pancreatic islets. Furthermore, groups II, III, and IV, as diabetic groups, showed a reduction in the intensity of brown granules, which was indicated by the decreased the number of insulin-positive cells (Figure 4b, c, d).

The present study develops a viable rat model induced by HFFD and low-dose STZ to mimic the pathophysiology of T2DM that should be metabolically stable, simple, affordable, and quick (Wickramasinghe et al., 2022). Many studies on this topic have recently varied regarding animal models, diet duration and composition, STZ dose, and pharmacological parameters assessed. Consequently, it is difficult to replicate the results and compare other diabetic models (Barrière et al., 2018; Gheibi et al., 2017; Wickramasinghe et al., 2022). However, it is still valuable to determine how to improve the HFFD-fed STZ-induced rat model by changing one or more factors to enhance its suitability (Wickramasinghe et al., 2022).

In our study, T2DM models were induced by HFFD for 2, 4, and 6 weeks before the STZ (35 mg/kg BW) injection and followed by this HFFD induction for 3 weeks. HFFD agents in our study consisted of a fat diet (2 ml of egg yolk and 2 ml of lard) and fructose (3.6 g/kg BW).

Table II. Insulin expression in the pancreatic islets

	Insulin-positive cells		Insulin-negative cells		Percentage of insulin-positive cells
	Total number in five islets	The average number per islet	Total number in five islets	The average number per islet	
Group I	346.333±130.059	69.267±26.012	207.000±24.214	41.400±4.843	59.157±7.312
Group II	193.667±39.633	38.733±7.927	374.667±103.161	74.933±20.632	36.006±10.263
Group III	104.667±47.688	20.933±9.538	325.000±48.993	65.000±9.799	22.733±7.071#
Group IV	115.333±14.111#	23.067±2.822#	265.000±59.702	53.000±11.940	31.628±3.949

All groups were compared with group I. Values are mean ± SEM, n = 3 samples per group. All values are $p < 0.05$ which was considered statistically significant. (#) a significant difference with group I. The percentage of insulin-positive cells is calculated as 100 x (values of insulin-positive cells/ values of insulin-positive cells + values of insulin-negative cells).

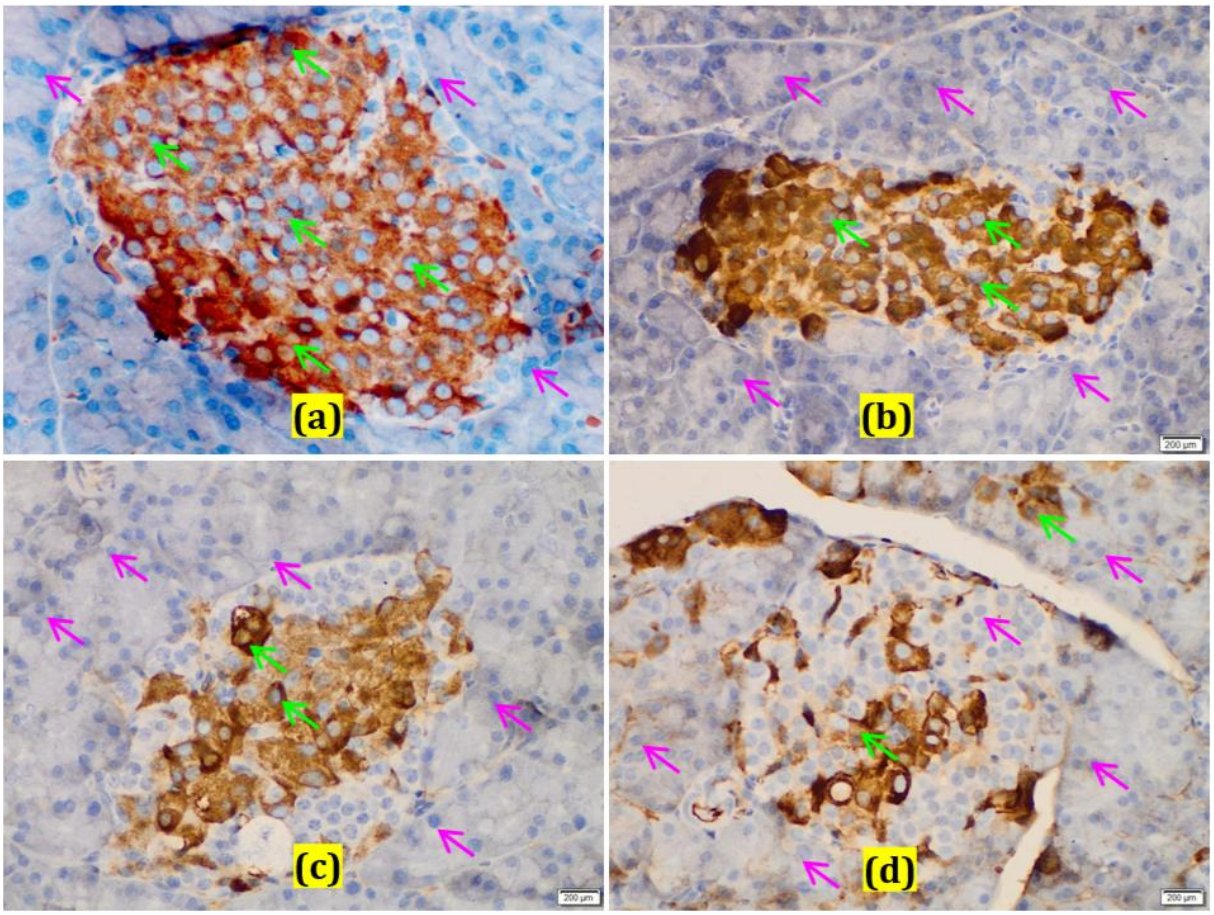

Figure 4. Photomicrographs of insulin immunohistochemical staining of pancreatic islets (400 x). (a) group I, (b) group II, (c) group III, and (d) group IV. (←): insulin-positive cells. (←): insulin-negative cells.

Higher egg consumption positively correlates with elevated human blood glucose and lipid profiles. In addition, a lard diet in rats promotes glucose, cholesterol, triglyceride, leptin, and TNF-α levels (Viggiano et al., 2016; X. Wang et al., 2019). Fructose at 40 g/day in humans (or 3.6 g/kg BW) can alter hepatic insulin sensitivity and lipid metabolism (Aeberli et al., 2013). On the other hand, high-dose STZ (45 and 55 mg/kg BW) causes severely impaired insulin secretion and leads to ketone body formation, which is similar to T1DM, whereas STZ (25 mg/kg BW) was similar to

normoglycemic conditions (Gheibi et al., 2017; Okoduwa et al., 2017). Other research designs have used multiple low-dose approaches. However, this method may cause more significant discomfort in animals and may influence the reproducibility of results due to an increased risk of injection procedure errors (Wickramasinghe et al., 2022). Therefore, in this study, a single low-dose was administrated.

This study revealed that T2DM inductors significantly increased BW, FBG, and triglyceride levels, but did not alter the FINS level. Previous studies showed that HFD, 20% fructose, and STZ (25, 35, 45, and 55 mg/kg BW, single doses) significantly increased BW, FBG, and triglyceride; and reduced serum insulin (Okoduwa et al., 2017). High BW correlated with HOMA-IR and obesity, thus increasing the risk of T2DM (Martinez et al., 2017). FBG is the primary biomarker for early DM detection and is initiated by the inability of tissues to respond to insulin appropriately. This is known as insulin resistance and pancreatic β-cell dysfunction (Chung et al., 2017; Lima et al., 2022). DM animal models need verification of FBG and stability studies (Gheibi et al., 2017; Guo et al., 2018). In this study, all treatment groups with DM conditions (>126 mg/dl) were detected; however, only group II had stability until 21 days after STZ injection compared with group I and day 0). Triglyceride is an independent risk factor, positive correlation, and predictor for T2DM (Zhao et al., 2019). Triglyceride values of all treatment groups in the range of borderline-high (150 – 199 mg/dl) were detected, and only group II had stability until 21 days after STZ injection in the range of borderline-high. On the other hand, one of the characteristics of late-stage T2DM is low insulinemia (Macho-González et al., 2020); meanwhile, no significant difference in this study ($p < 0.05$).

Two insulin predictors were measured, i.e., the TyG index and HOMA-IR. TyG index positively correlates with HOMA-IR and HbA1c (Selvi et al., 2021). The insulin resistance threshold is at 4.49 on the TyG index (Salazar et al., 2018); all induced groups on days 7, 14, and 21 after STZ injection showed a significant elevation above the threshold compared to day 0. In addition, HFD induction for 21 days, followed by STZ (35 mg/kg BW), showed a significant increase in the TyG index (Ghelani et al., 2017). HOMA-IR predicts insulin resistance with score ≥ 2.5 as a cut-off value (Park et al., 2021). Meanwhile, the normal and all induced groups were below the cut-off values. All induced groups showed significant elevation compared with day 0 on days 7 and 21 after STZ injection. In other studies, induction of HFD (22% fat, 48% carbohydrates, and 20% protein) for 4 weeks, followed by STZ (35 mg/kg BW), showed a significant increase in HOMA-IR values (Ghiasi et al., 2015).

T2DM also implicates kidney and liver functions (Makinde et al., 2020). A low level of creatinine and a high level of ALT represent abnormal of kidney and liver functions (Makinde et al., 2020; Pramono et al., 2018). Although T2DM is marked by low serum creatinine, diabetic animal models showed increased creatinine levels (Makinde et al., 2020). This study showed no significant changes in creatinine and ALT levels ($p < 0.05$). Another study showed HFD induction for 4 weeks, followed by STZ (35 mg/kg BW) showed an elevation of creatinine and ALT levels (Makinde et al., 2020).

Type 2 diabetic rats also have impaired organ index, such as the liver and kidney (Guo et al., 2018). Four organs were measured, including the pancreas, kidney, liver, and heart; meanwhile no significant difference compared with the normal group ($p < 0.05$). Another study revealed that HFD for 4 weeks followed by STZ at 35 mg/kg BW significantly elevated the liver and kidney index (Guo et al., 2018). The microscopic architecture of organs also influences T2DM, so the observed necrosis score (histopathology with HE stained) and percentage of insulin-positive cells (IHC). This study showed that the necrosis scores of the pancreas, kidney, and liver were elevated, whereas, there was no change in the necrosis of the heart. A previous study revealed that a high-sugar and HFD for 16 weeks followed by STZ at 35 mg/kg BW could increase the necrosis score in rats (Motshakeri et al., 2014). The pancreas showed that lowering insulin-positive cells and the percentage of insulin-positive cells elevates insulin-negative cells. Another study showed that an HFD of 40% for 4 weeks, followed by STZ (40 mg/kg BW), could decrease the percentage of insulin-positive cells in rats (Chandrasekaran et al., 2018).

Type 2 DM is related to blood glucose and lipid concentration (L. Wang et al., 2022). High blood glucose levels positively correlate with triglyceride, LDL, TG/HDL ratio, and LDL/HDL ratio (L. Wang et al., 2022). In this study, we measured the FBG, triglyceride, and TyG index for all treatments of DM rat models. All treatments showed abilities to enhance FBG, triglyceride, and

TyG index. Group II (HFFD-induced for 2 weeks before STZ injection) showed the most stability for FBG, triglyceride, and TyG index parameters. On the other hand, the study also measured FINS and HOMA-IR; meanwhile, there were no significant differences among all treatments. Therefore, insulin resistance conditions can be monitored through the TyG index (Salazar et al., 2018).

Type 2 DM models combine HFFD and low-dose STZ to influence the architecture of the pancreas (Suman et al., 2016; Udumula et al., 2021). All treatment groups showed normal and mild necrosis of pancreas architecture through H&E-stained histological examination. Furthermore, group III (HFFD-induced for 4 weeks before STZ injection) showed a lowering percentage of insulin-positive cells, and group IV (HFFD-induced for 6 weeks before STZ injection) showed a lowering number of insulin-positive cells. This study did not show necrosis of the pancreas; however, it has been able to increase glucose levels and reduce the percentage of insulin-positive cells. In this study, HFFD and low-dose STZ induction did not influence kidney function, involving creatinine, kidney index, and necrosis score of kidney architecture. Kidney data demonstrated a correlation that this study did not affect kidney functions or complications. This study also did not affect liver functions due to no change in ALT levels and liver architecture necrosis score. Therefore, this study did not influence liver functions in biochemical and histopathological analysis. Moreover, this study did not alter the heart index and necrosis of heart architecture. So, this study did not impact heart functions in type 2 DM rat models.

In this study, the induction of HFFD followed by STZ injection (35 mg/kg BW) successfully developed a type 2 DM rat model. Thus, an important marker for assessing glycemic and insulin resistance (FBG and TyG index). However, no glucose tolerance is a limitation of this study. The model should be further evaluated using a standard oral hypoglycemic drug, such as metformin to determine its appropriateness in examining the therapeutic efficacy as candidates of anti-DM agents.

CONCLUSION

In conclusion, this study showed that an initial feeding of the high-fat and high-fructose diet combination for 2 weeks followed by low-dose streptozotocin (35 mg/kg BW, i.p.) could produce efficient and stable characteristics of type 2 diabetes mellitus. This model is inexpensive and straightforward to develop and can be used to evaluate the effects of various antidiabetic drugs for treating type 2 diabetes mellitus.

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DECLARATIONS OF INTEREST

The authors declare that there were no conflicts of interest.

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