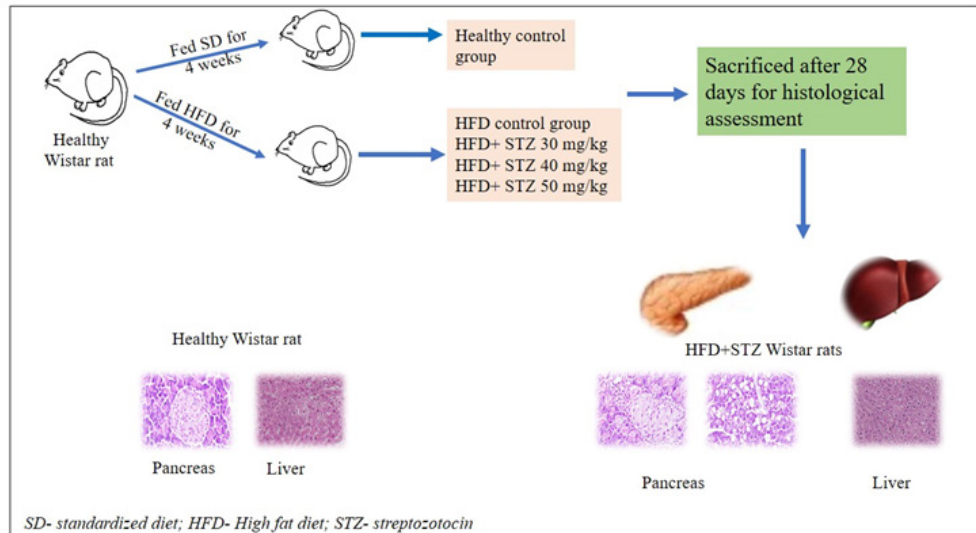


RESEARCH ARTICLE

Pancreatic and hepatic histopathology of high-fat diet fed streptozotocin-induced Wistar rat model of type 2 diabetes mellitus

A. S. D. Wickramasinghe, A. P. Attanayake*, T. W. Wijesiri, H. H. Peiris and L.K.B. Mudduwa



Highlights

- Prominent alterations were noted in the pancreatic and liver tissues of the Wistar rats fed with high-fat diet, followed by streptozotocin (50 mg/kg, ip).
- The liver showed no fatty change, except prominent hydropic degeneration in the hepatocytes.
- The major histopathological changes in the pancreas were loss of pancreatic islets, pancreatic islet hypertrophy, and mild fatty change in the exocrine pancreas.
- The established model could be useful in investigating antidiabetic mechanisms of novel pharmaceutical agents.

RESEARCH ARTICLE

Pancreatic and hepatic histopathology of high-fat diet fed streptozotocin-induced Wistar rat model of type 2 diabetes mellitus

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Abstract: In search of new fangled therapeutic agents against type 2 diabetes mellitus (T2DM), an appropriate preclinical disease model is important to ensure the success of subsequent clinical trials. There is an increasing trend in the use of high-fat diet (HFD) fed streptozotocin (STZ)-induced rodent models in investigating novel therapeutic agents against T2DM. Though several studies have been conducted on the development of the aforementioned model they were mainly focused on biochemical characteristics. Only limited studies have focused on histopathological changes which is the gold standard in investigating the treatment-related changes of novel therapeutic agents. Therefore, the present study aimed at assessing the histopathological changes of the pancreas and liver, the two main organs involved in glucose homeostasis, in a newly developed HFD-fed STZ-induced Wistar rat model. For this study, Wistar rats were induced with T2DM by intraperitoneal injection of STZ (30, 40, and 50 mg/kg) which was given after feeding the rats with an HFD consisting of 60% calories from fat for four weeks duration. Diabetic rats were sacrificed after maintaining for further four weeks. The liver and pancreas were excised and hematoxylin and eosin-stained sections were observed using a light microscope. The major histopathological changes in the pancreas of HFD-fed STZ-induced diabetic rats were loss of pancreatic islets, pancreatic islet hypertrophy, and mild fatty change in the exocrine pancreas. In contrast, the liver did not show any degree of fatty change, but the majority showed prominent hydropic degeneration in the hepatocytes. In conclusion, prominent alterations were well noted in the pancreatic and liver tissues of the Wistar rats fed with HFD, followed by STZ (50 mg/kg), and the established model could be useful in investigating and elucidating antidiabetic mechanisms of novel pharmaceutical agents.

Keywords: animal model, diabetes mellitus, histological changes, streptozotocin

INTRODUCTION

Globally, 537 million adults aged between 20-79 years are estimated to be surviving with diabetes mellitus (DM), and patients with DM are estimated to rise to 783 million by the year 2045. DM accounted for 747,000 deaths in South-East Asia Region alone in the year 2021 with 10.1 billion USD spent on diabetes-related expenditures. The prevalence of adults aged 20-79 years of age living with DM in Sri Lanka was 11.3% in 2021 (IDF, 2021). Among them, 90%

of the cases were of type 2 diabetes mellitus (T2DM), with hyperglycemia associated with insulin resistance and/or defects in insulin secretion. However, it is reported that there is a potential possibility of prevention, delaying, or remission of T2DM. Despite the available therapeutic options, the prevalence of T2DM is rising rapidly (IDF, 2021). Therefore, there is an increased trend of research on the discovery and development of new therapeutic agents for the prevention/treatment of T2DM.

In the search for novel therapeutic agents against T2DM, the use of a suitable animal model for preclinical investigations plays a vital role that will support the success of subsequent clinical trials. Rodents are commonly used as animal models of DM due to their short generation time and economic feasibility (Gheibi et al., 2017). Among them, streptozotocin-induced rats are reported to be one of the most commonly used rodent models in DM. Streptozotocin is a glucosamine-nitrosourea compound that destructs pancreatic β cells by DNA alkylation thereby producing hyperglycemia (Szkudelski, 2001). However, streptozotocin alone is unable to produce insulin resistance which is a key pathophysiological feature of T2DM (Bagaméry et al., 2020). Therefore, high-fat diet (HFD) fed streptozotocin (STZ)-induced rats have become popular in recent years as T2DM models (Chao et al., 2018). In such models, the HFD feeding will establish insulin resistance in rats and subsequent injection of STZ at an appropriate dose will cause partial β cell destruction simulating pathophysiological conditions much similar to T2DM (Gheibi et al., 2017).

There are several studies on the development of HFD-fed STZ-induced rodent models reported in the literature and most of the studies focused only on the biochemical characteristics of the model (Reed et al., 2000, Srinivasan et al., 2005, Okoduwa et al., 2017). However, a focus on histopathological analysis is also important in developing animal models of human diseases to more accurately evaluate the pathophysiological status of the model (Knoblauch et al., 2018). The pancreas and liver play vital roles in glucose homeostasis and are hence closely

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associated with the pathogenesis of T2DM. The key observations of histopathological examinations such as the degree of cell damage or fat accumulation in pancreatic and/or hepatic tissues of the disease model could provide better information, especially about the mechanisms of action of novel therapeutic agents against T2DM (Röder et al., 2016). The present study aimed to establish a detailed histopathological profile of the pancreas and liver tissue from a biochemically well-characterized HFD-fed STZ-induced rat model developed by our research team (Wickramasinghe et al., 2022). The established histopathological profile will be useful in elucidating the mechanisms of novel pharmaceutical agents using the developed HFD-fed STZ-induced Wistar rat model of T2DM.

MATERIALS AND METHODS

Chemicals and equipment

Streptozotocin was purchased from Sigma-Aldrich Corporation, USA. All the other chemicals and reagents were of analytical grade. A tissue processor (Shandon, UK), microtome (Thermo Fisher, Germany), and a light microscope (Olympus CX 21, Japan) were used in the preparation and observation of hematoxylin and eosin-stained sections of the pancreas and liver.

Experimental animals

Healthy male Wistar rats at 10 to 12 weeks of age and weighing 150 ± 15 g were procured from the Medical Research Institute (MRI), Colombo, Sri Lanka. The animals were housed in standard polystyrene cages and maintained at a controlled room temperature of $22-24^{\circ}\text{C}$ while providing 12-hour light-dark cycles in the animal house facility of the Faculty of Medicine, University of Ruhuna, Sri Lanka. Before dietary manipulation, all the animals were provided with a standard laboratory diet (2400 cal/g with 6% w/w fat, 16% w/w protein, and 68.2% w/w carbohydrates) and water *ad libitum*. The animals were acclimatized to the aforementioned controlled environmental conditions for one week before the initiation of the experiments.

Ethical considerations

Ethical approval for this study was granted by the Ethics Review Committee (ERC) of the Faculty of Medicine, University of Ruhuna, Sri Lanka under reference number

2020.P.004 (21.01.2020). Animal experiments were carried out according to the institutional standards established by the ERC and adhered to the guidelines of the Council of International Organizations of Medical Sciences (CIOMS) for animal research. The 3R principle was followed for all experiments. The experimental animals were handled carefully and anesthetized properly to ensure minimum distress to the animals.

Development of a high-fat diet-fed streptozotocin-induced diabetic rat model

The development of the diabetic model was conducted according to Furman (2021), with modifications. The rats were fed with HFD (3400 cal/g with 24.8% w/w fat, 54.6% w/w carbohydrate, and 12.8% w/w protein) containing 60% calories from fat which was prepared by adding butter (7400 cal/g with 55% saturated fat) as the fat source into the standard laboratory diet. After feeding HFD for four weeks, T2DM was induced by an injection of STZ (30, 40, and 50 mg/kg, ip) (Furman, 2021). The fasting serum glucose concentrations of experimental rats were determined using the enzymatic colorimetric method (GOD-PAP) with a spectrophotometric assay kit, 72 hours post-injection of STZ (Trinder, 1969). The hyperglycemic rats (i.e. rats with fasting plasma glucose concentrations ≥ 11.1 mmol/L) were selected for further studies (Furman, 2021).

Study design

For this study, the HFD-fed STZ-induced diabetic rats were randomly grouped into three groups ($n = 10/\text{group}$), STZ30; HFD-fed rats injected with STZ (30 mg/kg, ip), STZ40; HFD-fed rats injected with STZ (40 mg/kg, ip), STZ50; HFD-fed rats injected with STZ (50 mg/kg, ip). Apart from that, two control groups ($n = 10/\text{group}$) were maintained as healthy control; STZ uninjected rats provided with a standard laboratory diet and HFD control; STZ uninjected rats provided with the HFD. The study design is depicted in Figure 1. The control group rats and diabetic animals were maintained for further 28 days providing respective diets. Animals were sacrificed on the 29th day of the study and pancreatic and liver tissues were excised for histopathological examination.

Histopathological assessment

The excised pancreatic and hepatic tissues were fixed in 10% buffered formalin in wide-mouth, leak-proof containers. They were transferred to labeled tissue

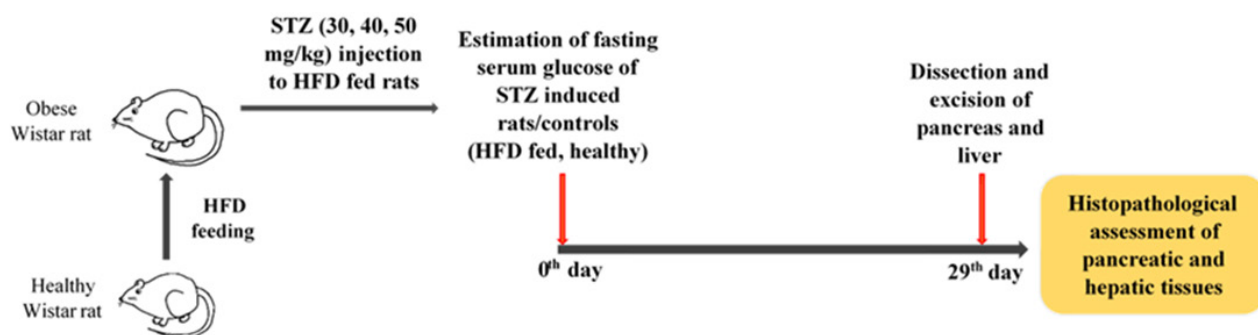


Figure 1: Study design for the development of high-fat diet fed-streptozotocin-induced Wistar rat model
HFD; high-fat diet, STZ; streptozotocin

cassettes, and processed, using a tissue processor, and the processed tissues were embedded in paraffin. The tissue blocks were trimmed in a microtome at 10 µm thickness and cut into sections of 5 µm thickness. Afterward, the tissue sections were deparaffinized, hydrated, and stained with hematoxylin and eosin (H & E). H & E stained tissue sections were then observed for histological changes under a light microscope, by three independent investigators, which included a consultant histopathologist and the investigators were blinded to the experimental groups.

The pancreatic sections were examined for morphological changes which included loss of pancreatic islets, pancreatic islet hypertrophy, and fatty change in the exocrine pancreas. The loss of pancreatic islets was scored using an adapted score system from a previous study in which the score ranged from 0 to 3 (0= Normal (29 average number of islets per section), 1= Mild (loss of 1/3 of islets), 2= Moderate (loss of 1/3 to 2/3 islets), 3= Severe (loss of >2/3 of islets)) (Attanayake et al., 2016). The appearance of hypertrophic pancreatic islets was scored adapting the same score system and ranged from, 0 to 3 (0= No hypertrophic islets, 1= 1/3 of pancreatic islets are hypertrophic, 2= 1/3-2/3 of pancreatic islets are hypertrophic, 3= >2/3 of pancreatic islets are hypertrophic). The entire section of the pancreas was examined for counting pancreatic islets at ×100 magnification.

Fatty change in the exocrine pancreas was examined in 20 adjacent high power (×400) microscopic fields and scored 0-4 (0= None, 1= Mild (<10% of exocrine pancreas with fatty change), 2= Moderate (10-30% of exocrine pancreas with of fatty change), 3= Marked (31-60% of exocrine pancreas with fatty change), 4= Severe (>60% of exocrine pancreas with fatty change)) according to a scoring system adapted from a previous study (Niazi et al., 2021).

The morphological changes in the rat liver (lobular inflammation, and hepatocyte hydropic degeneration) were also observed. Lobular inflammation and hepatocyte hydropic degeneration were scored according to the Brunt

scoring system. The lobular inflammation was scored 0-3 (0= Absence of the feature, 1= Mild (<2 foci/200x field), 2= Moderate (2-4 foci/200x field), 3= Severe (>4 foci/200x field) and hepatocyte hydropic degeneration was scored 0-2 (0= Absence, 1= Minimal hydropic degeneration, 2= Prominent hydropic degeneration) in the Hematoxylin and Eosin (H & E) sections (Kleiner et al., 2005).

Statistical analysis

Histopathology score values were presented as mean scores per each group. The Kruskal-Wallis test was used to analyze semi-quantitative data of histopathological scores. A $p < 0.05$ was considered statistically significant.

RESULTS

Histopathological changes of the pancreas

Histomorphological features in H & E-stained pancreatic tissues of rats are presented in Figure 2. The pancreatic islets of healthy rats were normal in size with well-demarcated borders. HFD feeding resulted in pancreatic islet hypertrophy. Subsequent STZ injection caused mild to severe loss of pancreatic islets (Table 1). However, the increase in number of pancreatic islets with hypertrophy was also evident in STZ-induced rats (Table 2). The hypertrophic pancreatic islets of STZ-induced rats had irregular borders in contrast to HFD group rats where the hypertrophic pancreatic islets were elongated in shape with regular borders (Figure 2). STZ injection also resulted in a mild fatty change in the exocrine pancreas (Table 1, Figure 3). Apart from that, fatty changes in the exocrine pancreas were also presented in the HFD-fed group and STZ-induced group.

Histopathological alterations in the liver

Figure 4 depicts the liver tissues of the healthy control rats demonstrating normal hepatocyte architecture. The major histopathological alteration observed in liver tissues of STZ-induced diabetic rats was hydropic degeneration of

Table 1: Histomorphological changes of the pancreas

Animal Group	Score values		
	^a Loss of pancreatic islets	^b Hypertrophic pancreatic islets	^c Fatty change in the exocrine pancreas
HC	0.00	0.00	0.00
HFD	1.33	1.00	0.00
STZ30	1.67	1.00	0.07
STZ40	2.00	1.00	0.18
STZ50	3.00*	2.00*	1.00*

Score values are mentioned as mean.

^a0= Normal (29 average no. of islets per section), 1= Mild (loss of 1/3 of islets), 2= Moderate (loss of 1/3 to 2/3 islets), 3= Severe (loss of >2/3 of islets)

^b0= No hypertrophic islets, 1= 1/3 of pancreatic islets are hypertrophic, 2= 1/3-2/3 of pancreatic islets are hypertrophic, 3= >2/3 of pancreatic islets are hypertrophic

^c0= None, 1= Mild (>10% of exocrine pancreas with fatty change), 2= Moderate (10-30% of exocrine pancreas with fatty change), 3= Marked (31-60% of exocrine pancreas with fatty change), 4= Severe (>60% of exocrine pancreas with fatty change)

HC; healthy control, HFD; high-fat diet, STZ30; streptozotocin (30 mg/kg), STZ40; streptozotocin (40 mg/kg), STZ50; streptozotocin (50 mg/kg)

*Statistically significant ($p < 0.05$) compared to the healthy control group.

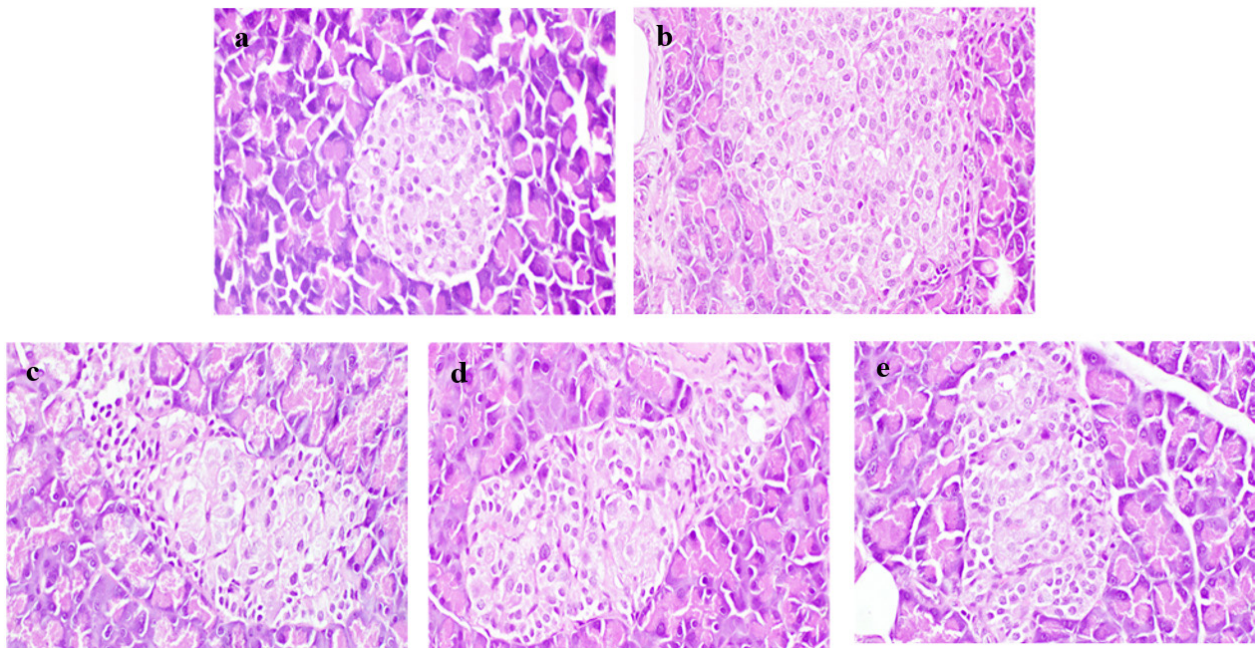


Figure 2: Histopathological changes in the pancreatic islets in high-fat diet-fed streptozotocin-induced diabetic Wistar rat model ($\times 400$) (a): A normal pancreatic islet of healthy Wistar rats, (b): Hypertrophic pancreatic islet with regular borders in the high-fat diet fed control group rats, Hypertrophic pancreatic islets with irregular borders in (c): high-fat diet fed streptozotocin (30 mg/kg)-induced rats, (d): high-fat diet fed streptozotocin (40 mg/kg)-induced rats, and (e): high-fat diet fed streptozotocin (50 mg/kg)-induced rats

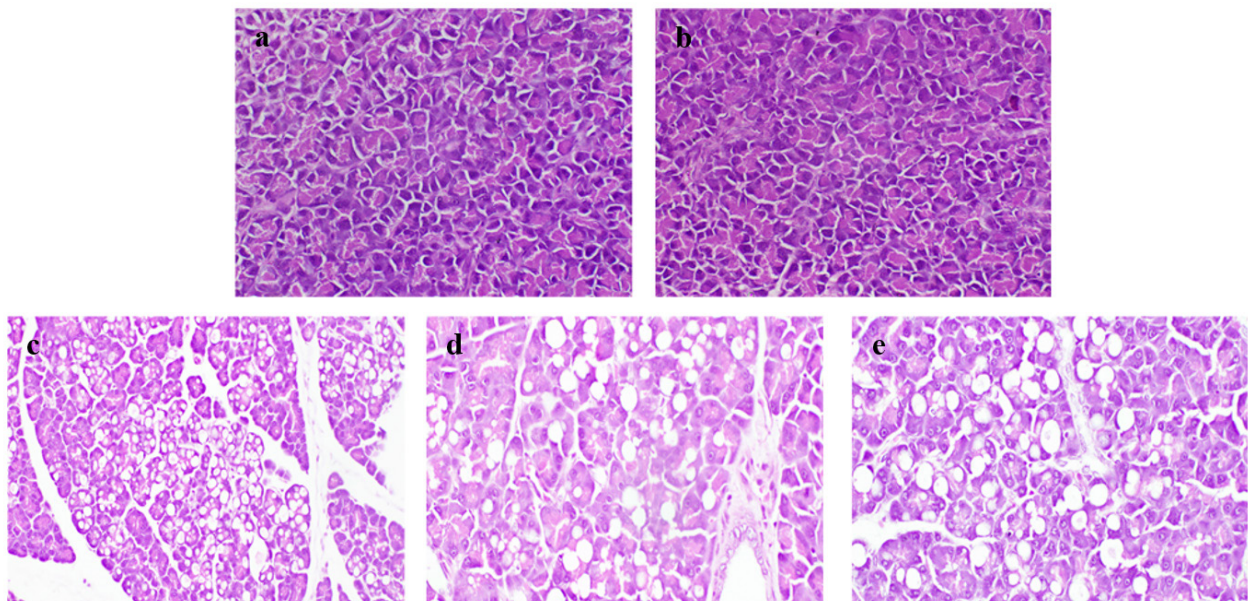


Figure 3: Histopathological changes in the exocrine pancreas in high-fat diet-fed streptozotocin-induced diabetic Wistar rat model. Normal exocrine pancreatic architecture of (a) healthy Wistar rats ($\times 400$), (b) high-fat diet fed control group rats ($\times 400$), Fatty change of exocrine pancreas in (c) high-fat diet fed streptozotocin (30 mg/kg)-induced rats ($\times 100$), (d) high-fat diet fed streptozotocin (40 mg/kg)-induced rats ($\times 400$), and (e) high-fat diet fed streptozotocin (50 mg/kg)-induced rats ($\times 400$)

hepatocytes (Figure 5). According to semi-quantitative analysis, there was no significant difference ($p > 0.05$) in hepatocyte hydropic degeneration in STZ (30 mg/kg and 40 mg/kg) induced groups compared to the healthy control group. However, a significant increase ($p = 0.001$)

in hepatocyte hydropic degeneration was observed in the liver tissues of the STZ (50 mg/kg) induced group (Table 2). Lobular inflammation was gradually reduced with the increasing dose of STZ, however, the change was not statistically significant (Figure 6).

Table 2: Histomorphological changes in the liver

Animal group	Average Score values	
	Lobular inflammation	Hepatocyte hydropic degeneration
HC	0.00	0.00
HFD	0.60	1.67
STZ30	0.80	0.83
STZ40	0.50	0.50
STZ50	0.33	2.17*

Score values are mentioned as mean.

Definition of scores:

Lobular inflammation: 0= Absence of the feature, 1= Mild (<2 foci/200x field), 2= Moderate (2-4 foci/200x field), 3= Severe (>4 foci/200x field)

Hepatocyte hydropic degeneration: 0= Absence, 1= Minimal hydropic degeneration, 2= Prominent hydropic degeneration

HC; healthy control, HFD; high-fat diet, STZ30; streptozotocin (30 mg/kg), STZ40; streptozotocin (40 mg/kg), STZ50; streptozotocin (50 mg/kg)

*Statistically significant ($p < 0.05$) compared to the healthy control group.

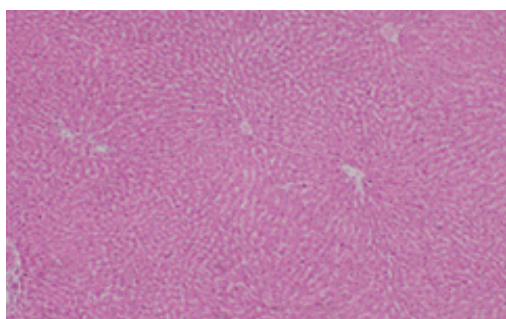


Figure 4: H&E stained section of the liver in healthy Wistar rats ($\times 100$)

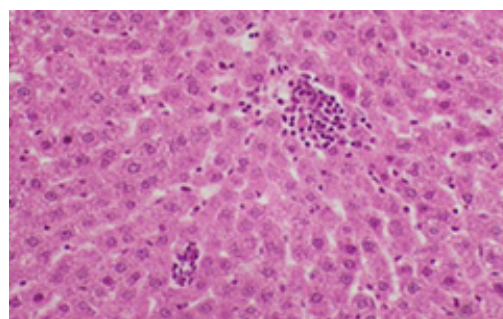


Figure 6: Lobular inflammation in H & E (Hematoxylin and Eosin) stained liver sections of high-fat diet-fed control rats ($\times 400$)

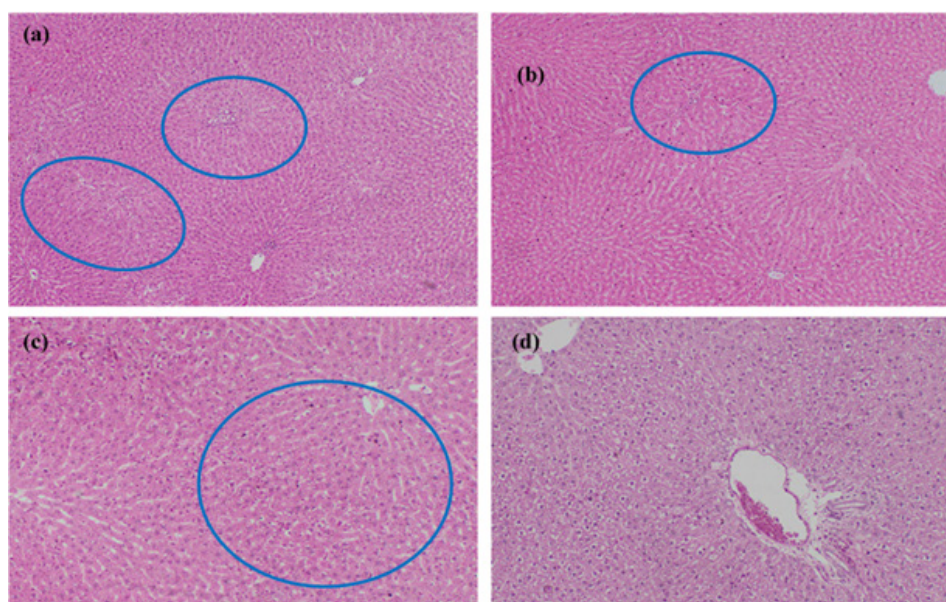


Figure 5: Degree of hydropic degeneration of H & E (Hematoxylin and Eosin) stained liver sections of high-fat diet fed streptozotocin-induced diabetic Wistar rat model. (a) High-fat diet fed control rat (prominent hydropic degeneration) ($\times 100$), (b) High-fat diet fed streptozotocin (30 mg/kg)-induced rats (minimal hydropic degenerations) ($\times 100$), (c) High-fat diet fed streptozotocin (40 mg/kg)-induced rats (minimal hydropic degenerations) ($\times 100$), (d) High-fat diet fed streptozotocin (50 mg/kg)- induced rats (prominent hydropic degeneration) ($\times 100$). Circles in a, b, and c highlight the area of hydropic degeneration in zone 1.

DISCUSSION

The pancreas and liver are the vital organs involved in the regulation of blood glucose. The pancreas is mainly involved in secreting insulin and glucagon hormones in response to blood glucose levels. The liver maintains glucose homeostasis via storing glucose as glycogen (glycogenesis) or releasing glucose to the bloodstream via the synthesis of glucose (gluconeogenesis) or breakdown of glycogen (glycogenolysis) in the presence of insulin or glucagon, respectively (Röder et al., 2016). Previous studies on the development of HFD-fed STZ-induced diabetic rat models focus mainly on biochemical changes. Only one study on animal model development focused on histopathological changes in the pancreas and liver in addition to the biochemical parameters (Guo et al., 2018). However, focusing on histopathological changes in the pancreas and liver is important, especially when investigating the antidiabetic mechanisms of novel therapeutic agents. For example, growing evidence suggests that there is a reduced β cell mass in T2DM patients at least in the later stages of the disease. In T2DM, a progressive decline in the number of pancreatic islets and β cell mass is suggested to occur due to the exhaustion of β cells in an attempt to compensate for the insulin requirement imposed by insulin resistance. Furthermore, de-differentiation of β cells is also reported in patients with T2DM (Wang et al., 2021). Therefore, substances causing β cell regeneration or re-differentiation of β cells could be beneficial as therapeutic agents against T2DM where histopathological analysis is essential to observe such effects. In the current study, the pancreas from the HFD and HFD-fed STZ-induced diabetic rats demonstrated pathological changes in both exocrine and endocrine components. Therefore, a T2DM model with prominent pathological changes in the pancreatic architecture is beneficial for evaluating the activity of novel therapeutic agents in terms of improving the pancreatic architecture. In the present study, the hypertrophic islets observed upon HFD feeding could be due to the attempt of the pancreatic β cells to compensate for the insulin demand imposed by insulin resistance. Similar observations are reported in another study where an increase of islet area is reported to occur with feeding HFD consisting of 60% fat for four weeks (Ickin et al., 2015). Furthermore, STZ-induced compensatory pancreatic islet hypertrophy along with damage to pancreatic islets has been observed in HFD-fed rats injected with STZ in a previous study, which is in agreement with the findings of the present study (Chen et al., 2022). In HFD-fed STZ-induced rats, it is assumed that the β cells secrete large amounts of insulin during the compensatory mechanism; however, as the disease progresses, this β -cell function deteriorates, leading to a deterioration in glucose homeostasis (Lebovitz & Banerji, 2004). Furthermore, the number of islet cells; precisely the number of β cells in the pancreatic islets determines the total amount of insulin released (Chen et al., 2017). The damage to the pancreatic islets that occurred upon subsequent STZ injection was manifested by characteristics such as irregular pancreatic islet borders and decreased number of pancreatic islets that were in agreement with previous studies conducted on the

investigation of therapeutic compounds using the HFD-fed STZ-induced rodent models (Zhou et al., 2009, Antony et al., 2017, Jiao et al., 2017, Oza and Kulkarni, 2020, Chen et al., 2022). The exact histomorphological changes and islet cell mass regulation differ between humans and rodents, however, animal models have been widely used in the investigation of antidiabetic mechanisms of novel drugs due to the limitation of the availability of human samples and the lack of technologies to investigate human islet cell biology (Noordin et al., 2021). The fatty change in the exocrine pancreas in HFD-fed rats upon STZ injection as observed in this study could be attributed to the increased free fatty acid concentration in a hyperglycemic condition (Rugivarodom & Geeratrugool, 2022). A fatty change of a similar nature is reported in another study with T2DM rats (Noordin et al., 2021). Hence, the fatty change in the exocrine pancreas observed in this animal model can also be a useful marker for investigating the amelioration effect of T2DM by antidiabetic agents. Moreover, pancreatic lipid content represented by a fatty change in the exocrine pancreas may contribute to the dysfunction of islet cells and possibly to the onset and subsequent development of complications of T2DM (Skovsø, 2014).

The histopathological changes observed in the liver tissue of HFD-fed STZ-induced rats in this study include hydropic degeneration of hepatocytes and mild lobular inflammation. In previous studies, HFD-fed STZ-induced rat models are reported to have fatty liver and histopathological changes including lipid accumulation and lobular inflammation (Guo et al., 2018, Dwivedi & Jena, 2020). However, in this study, any degree of fatty change in the liver was not observed in any of the study groups. The prolonged duration of HFD feeding (8-17 weeks) attributed to the development of fatty change in previous reports however, HFD-fed duration of the present study was only for 4 weeks. It is reported that the hepatic fatty changes were more pronounced in HFD-fed Wistar rats after around 8 weeks of feeding (Majo et al., 2023). Further, there was no significant association of lobular inflammation with an increase in hepatocyte hydropic degeneration.

HFD feeding followed by STZ (50 mg/kg) induced significant changes in pancreatic histology including loss of pancreatic islets, increased islet hypertrophy, and increased fatty changes in the exocrine pancreas with the significant increase in hepatocyte hydropic degeneration compared to healthy control. The histopathological changes are in agreement with body weight, food/calorie and water intake, and biochemical parameters of the same rats published previously (Wickramasinghe et al., 2022). In brief, HFD-fed STZ-induced rats manifested a significant increase in water intake indicating polydipsia a classical sign of the development of hyperglycemia, however with a slight increase in food and calorie consumption indicating increased energy expenditure. The persistence of body weight comparable to healthy control rats upon STZ injection indicated the development of type 2 DM as a drastic reduction of body weight upon STZ injection is usually observed in the development of type 1 diabetes. Moreover, STZ-induced rats were presented with hyperglycemia and increased glucose intolerance.

Homeostatic model assessment-Insulin resistance (HOMA-IR) was above 2.5 indicating insulin resistance and Homeostatic model assessment- β cell function (HOMA- β) was below 50% indicating impaired β cell function. The serum triglycerides (TG), total cholesterol (TC), very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol LDL-C were all significantly high indicating dyslipidemia. The magnitude of all biochemical parameters was significantly high in HFD-fed STZ (50 mg/kg) injected rats (Wickramasinghe et al., 2022).

In summary, the present study revealed that the STZ (50 mg/kg) dose produced more prominent biochemical and histopathological alterations in HFD-fed rats compared to STZ 30 and 40 mg/kg injected rats indicating the benefit of using STZ (50 mg/kg) dose in developing T2DM rat model. Though several studies reported the use of STZ doses below 50 mg/kg in developing the model, the studies have drawbacks including low successful rate and long-term stability of the models or unavailability of data on those aspects which were also highlighted previously (Wickramasinghe et al., 2022).

There are certain limitations in the present study. Monitoring the model for a longer time and examining morphological changes at different time points could provide more information about the model. In addition, extensive cellular molecular-level research is needed to uncover the underlying mechanisms of the recovery of the model.

CONCLUSION

The diabetic model developed by giving a single intraperitoneal injection of streptozotocin at a dose of 50 mg/kg to Wistar rats fed with a high-fat diet rich in saturated fat for a week, would closely simulate the histomorphological abnormalities of the pancreatic architecture as found in T2DM. Furthermore, the said diabetic model manifests T2DM-associated liver damage via hepatocyte damage and changes in hepatic morphology.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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