Integrative analysis on the effects of streptozotocin in the experimental model for diabetization in wistar rats: histological and laboratory study

Análise integrativa dos efeitos da streptozotocina no modelo experimental para diabetização em ratos wistar: estudo histológico e laboratorial

Análisis integrativo de los efectos de la estreptozotocina en el modelo experimental de diabetización en ratas wistar: estudio histológico y de laboratorio

DOI:10.34119/bjhrv7n2-171

Originals received: 02/19/2024
Acceptance for publication: 03/08/2024

Jaqueline Meert Parlow
Graduating in Medicine
Institution: Universidade Estadual de Ponta Grossa
Address: Avenida Carlos Cavalcanti, 4748, Ponta Grossa – PR, CEP: 84030-900
E-mail: parlowjaqueline@hotmail.com

Ana Cristina Barth de Castro
Graduating in Medicine
Institution: Universidade Estadual de Ponta Grossa
Address: Avenida Carlos Cavalcanti, 4748, Ponta Grossa – PR, CEP: 84030-900
E-mail: 19178040@uepg.br

Fabio Vinicius Barth
Graduating in Medicine
Institution: Universidade Estadual de Ponta Grossa
Address: Avenida Carlos Cavalcanti, 4748, Ponta Grossa – PR, CEP: 84030-900
E-mail: 19376240@uepg.br

Diego José Schebelski
Doctor in Pharmaceutical Sciences
Institution: Universidade Estadual de Ponta Grossa
Address: Avenida Carlos Cavalcanti, 4748, Ponta Grossa – PR, CEP: 84030-900
E-mail: diego.ski@hotmail.com

Rosana Letícia Rosa
Doctor in Pharmaceutical Sciences
Institution: Universidade Estadual de Ponta Grossa
Address: Avenida Carlos Cavalcanti, 4748, Ponta Grossa – PR, CEP: 84030-900
E-mail: rosanaleticia@hotmail.com
ABSTRACT
The study of complications of diabetes mellitus is important to understand the alterations on the specific tissues affected by this chronic disease. An animal model that simulated this complication is necessary for further treatments and prevention of diabetes complications. The aim of this study was evaluated the biochemical and histological alterations on liver, kidney, small gut and retina tissues. The animal assay was made with 15 wistar rats divided in two groups: a control group, consisting of 5 rats that were healthy and non-diabetic (CONTROL), and a streptozotocin group (STZ), consisting of 10 rats who were diabetic. A dosage of streptozotocin at a concentration of 40 mg/kg was administered intraperitoneally. Rats with glycemia levels below 150 mg/dl received a subsequent injection of 40 mg/kg of streptozotocin, whereas rats with glycemia levels ranging from 150 to 250 mg/dl were given a subsequent injection of 20 mg/kg of streptozotocin. The biochemical results showed difference in triglyceride, AST and ALT levels between the groups. The histological analysis of liver, kidney, small gut and retina showed significant alterations related to the chronic diabetes complications. These results suggested that this animal model can be used for future studies of metabolics mechanisms to understand and avoid the complications of diabetes.

Keywords: animal model, diabetes, insulin, metabolic alterations.

RESUMO
O estudo das complicações do diabetes mellitus é importante para compreender as alterações nos tecidos específicos afetados por esta doença crônica. Um modelo animal que simule esta complicação é necessário para tratamentos adicionais e prevenção de complicações do diabetes. O objetivo deste estudo foi avaliar as alterações bioquímicas e histológicas nos tecidos do fígado, rim, intestino delgado e retina. O ensaio animal foi feito com 15 ratos wistar divididos em dois grupos: um grupo controle, composto por 5 ratos saudáveis e não diabéticos (CONTROLE), e um grupo estreptozotocina (STZ), composto por 10 ratos diabéticos. Uma dosagem de estreptozotocina na concentração de 40 mg/kg foi administrada por via intraperitoneal. Ratos com níveis de glicemia abaixo de 150 mg/dl receberam uma injeção subsequente de 40 mg/kg de estreptozotocina, enquanto ratos com níveis de glicemia variando de 150 a 250 mg/dl receberam uma injeção subsequente de 20 mg/kg de estreptozotocina. Os resultados bioquímicos mostraram diferença nos níveis de triglicerídeos, AST e ALT entre os
grupos. A análise histológica do fígado, rim, intestino delgado e retina mostrou alterações significativas relacionadas às complicações crônicas do diabetes. Estes resultados sugerem que este modelo animal pode ser utilizado em estudos futuros de mecanismos metabólicos para compreender e evitar as complicações do diabetes.

**Palavras-chave:** modelo animal, diabetes, insulina, alterações metabólicas.

**RESUMEN**
El estudio de las complicaciones de la diabetes mellitus es importante para comprender las alteraciones en los tejidos específicos afectados por esta enfermedad crónica. Es necesario un modelo animal que simule esta complicación para tratamientos posteriores y la prevención de las complicaciones de la diabetes. El objetivo de este estudio fue evaluar las alteraciones bioquímicas e histológicas en los tejidos del hígado, riñón, intestino delgado y retina. El ensayo en animales se realizó con 15 ratas wistar divididas en dos grupos: un grupo control, formado por 5 ratas sanas y no diabéticas (CONTROL), y un grupo de estreptozotocina (STZ), formado por 10 ratas diabéticas. Se administró por vía intraperitoneal una dosis de estreptozotocina a una concentración de 40 mg/kg. Las ratas con niveles de glucemia inferiores a 150 mg/dl recibieron una inyección posterior de 40 mg/kg de estreptozotocina, mientras que las ratas con niveles de glucemia entre 150 y 250 mg/dl recibieron una inyección posterior de 20 mg/kg de estreptozotocina. Los resultados bioquímicos mostraron diferencias en los niveles de triglicéridos, AST y ALT entre los grupos. El análisis histológico de hígado, riñón, intestino delgado y retina mostró alteraciones significativas relacionadas con las complicaciones de la diabetes crónica. Estos resultados sugirieron que este modelo animal puede usarse para futuros estudios de mecanismos metabólicos para comprender y evitar las complicaciones de la diabetes.

**Palabras clave:** modelo animal, diabetes, insulina, alteraciones metabólicas.

**1 INTRODUCTION**

Diabetes mellitus (DM) is recognized as a metabolic syndrome, in which persistent hyperglycemia and one alteration in carbohydrate, lipid, and protein metabolism are characterized by a deficiency in insulin production and/or impaired insulin action\(^1\). The occurrence of vascular problems, caused from a series of metabolic alterations, is responsible for the development of several illnesses associated with diabetes mellitus, including nephropathy, neuropathies, retinopathies, and endocrine disorders\(^2\).

The predicted global population of diabetic adults, aged 20 to 79 years, was roughly 537 million in the year 2021. According to the International Diabetes Federation (2021), projections indicate an increase to 643 million by the year 2030, and further rise to 783 million by 2045\(^2\). The study of metabolic alterations of DM is important and animal models induced by the chemical streptozotocin (STZ) might offer valuable insights into the histological and laboratory alterations associated with this condition. These findings contribute to the overall comprehension and investigation of the underlying processes involved in this particular
disease³.

STZ is a glucosamine-nitrosourea compound that exhibits a strong attraction to pancreatic beta cells, mostly attributed to its structural resemblance to glucose⁴. The STZ do one fast and fatal reduction in the levels of nicotinamide adenine dinucleotide (NAD) within cells. This depletion subsequently results in a decline in adenosine triphosphate (ATP) levels, ultimately inhibiting the synthesis and secretion of insulin⁵. Furthermore, it has been observed that STZ administration induces the generation of free radicals, which have been implicated in the pathogenesis of diabetes⁶.

Previous studies conducted on animal models have examined the effects of STZ in inducing diabetes over a relatively short duration. These investigations have seen the development of hyperglycemia, which is compatible with the diabetic state, as well as changes in the lipid profile that resemble those observed in human individuals with diabetes. In addition to an elevation in inflammatory cytokines and reactive oxygen species (ROS)⁷. These observations support the proposition that animal models of diabetes mellitus produced via the administration of streptozotocin can bring significant insights into histology and laboratory alterations. With an extended duration of observation in this particular model, there is the potential for enhanced comprehension about the underlying processes associated with this illness³,⁸.

In the literature there is a great diversity of protocols used for diabetic rat models, in this way, the aim of the present study was to evaluate the model of diabetic rats in different systems, validating the protocol used.

2 METHODOLOGY AND EXPERIMENTAL DESIGN
2.1 ANIMAL ASSAY

Fifteen female Wistar rats (*Rattus norvegicus*) aged around 90 days were obtained from the Central Biotechnology of the State University of Ponta Grossa (UEPG) protocol number 21.000031633-4. The rats had a mean weight of 324 ± 52.65 g. Water and commercial feed were given in an environment with a temperature of 22 ± 2 °C, controlled humidity, and an autonomously regulated 12-hour light/dark cycle. The experimental protocol underwent prior submission for approval by the Ethical Committee for the Use of Animals at UEPG.

The animals utilized in the current study were subjected to randomization and afterwards separated into two distinct experimental groups: a control group, consisting of 5 rats that were healthy and non-diabetic (CONTROL), and a streptozotocin group (STZ), consisting of 10 rats who were diabetic. The animals were confined into boxes, with a maximum of five
animals. The boxes were equipped with environmental enrichment.

2.2 ESTABLISHMENT OF EXPERIMENTAL DIABETES MELLITUS

The animals were subjected to a 4-hour fasting period and then the capillary blood glucose levels were assessed using an ACCU-CHEK digital glucometer manufactured by Roche. Subsequently, a dosage of streptozotocin at a concentration of 40 mg/kg was administered intraperitoneally after dilution in saline solution. The control group received an equivalent volume of saline solution. On the second day of the experiment, the blood glucose levels of the rats were reassessed. Rats with glycemia levels below 150 mg/dl received a subsequent injection of 40 mg/kg of streptozotocin, whereas rats with glycemia levels ranging from 150 to 250 mg/dl were given a subsequent injection of 20 mg/kg of streptozotocin. No further administration of streptozotocin was conducted in rats exhibiting glycemia levels exceeding 250 mg/dl. Capillary insulin was measured every two days, animals with insulin above 500 mg/dl were administered 3 IU of insulin glargine.

2.3 EUTHANASIA AND TISSUE REMOVAL

Following an 10-week duration, all rats were subjected to euthanasia by the administration of an overdose of ketamine hydrochloride and xylazine. The weight and capillary blood glucose levels of the animals were assessed. The eyes, liver, kidneys, retroperitoneal and omental adipose tissue, and 10 cm segments of small intestine fragments (duodenum, jejunum, and ileum) were extracted and subsequently preserved in a 10% formalin solution for subsequent histological processes. After weighted the relative proportion of adipose tissue in each sample was analyzed in relation to the overall body weight of each individual animal.

2.4 LABORATORY ANALYSIS

The blood sample was obtained and centrifuged at a speed of 2500 RPM during 10 minutes. The serum samples were aliquoted into 2 mL tubes and stored under refrigeration at a temperature of -80°C. The measurement of cholesterol and triglyceride levels in serum was conducted using a colorimetric enzymatic (Ebram Produtos Laboratorios Ltda®) on a semi-automatic biochemical analyzer (BIO PLUS 200). The serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using a kinetic enzymatic kit (Ebram Produtos Laboratorios Ltda®) with the same equipment.

2.5 HISTOLOGICAL ANALYSIS
Following fixation in formalin 10%, the samples underwent dehydration, clearing, and paraffin embedding. Subsequently, sections of 5 µm in thickness were obtained using a microtome and subjected to staining with hematoxylin and eosin (HE). Subsequently, the histological sections were captured and analysed with an Olympus DP72 microscope equipped with the CellSens Standard application. The analyses were conducted in duplicate.

The Image J Software was utilized to assess the dimensions of the villi and the depth of the intestinal crypts. The study was conducted on a total of three samples, from which the mean and standard deviation (SD) were analysed.

The histological examination of the liver and kidney tissue was a qualitative assay to identify the presence of diabetic alterations. The morphometric study of the retina was conducted using the methodology outlined by Hughes. The study involved the measurement of the thickness of four distinct retinal layers, namely the thickness from the inner limiting membrane to the outer limiting membrane (ILM-OLM), the inner plexiform layer (IPL), the inner nuclear layer (INL), and the outer nuclear layer (ONL).

In order to assess the extent of retinal cell loss, a method was employed whereby the number of cell nuclei per 50 µm length in retinal slices was counted, yielding linear cell densities. The quantification of cell nuclei within the retinal layers (Ganglion Cell Layer, Inner Nuclear Layer, and Outer Nuclear Layer) was conducted in both hemispherical slices of the retina. This analysis was performed at a distance of 1.5 mm from the optic nerve head, with a specific focus on a 50 µm width region.

2.6 STATISTICAL ANALYSIS

The statistical analysis was conducted using the R Foundation for Statistical Computing version 4.3.0 software. The paired t-test was employed for sample groups that followed a normal distribution, while the Wilcoxon test was used for sample groups that did not meet the assumptions of normality. A confidence interval of 95% was utilized, with a significance level set at p < 0.05. The Shapiro-Wilk test was employed to examine the distribution of the samples within the population.

3 RESULTS AND DISCUSSION

3.1 GENERAL ANALYSIS

In relation to the animals body weight, the STZ group exhibited an average weight of 339.2 grams at the beginning and 299.2 grams at the end of the animal assay. The control group had average weights of 293.6 grams at the beginning and 422.4 grams at the end. Animals who
received STZ exhibited a statistically significant decrease (p < 0.05) during the course of the study, on the contrary the control group exhibited a statistically significant increase (p < 0.05) in body weight as shown in Figure 1. The aggression on the endogenous β cells subsequent to the onset of diabetes decrease the synthesis of insulin, increase of the hyperglycemia and an increased state of catabolism.

Figure 1– Comparison of body weight between the initial and final period. A) STZ Group. B) Control group.

The capillary blood glucose assay showed as depicted in Figure 2 that the average blood glucose level exhibits an increase in comparison to the control group. Prior to the initiation of the diabetes induction procedure, both groups of rats had blood glucose levels that did not display statistically significant difference, with values below 100 mg/dL. Following the process of induction, it was observed that all rats within the STZ group had blood glucose levels above 250 mg/dL. During the follow-up period, the blood glucose levels of the five control animals remained within the usual range for their respective species. In contrast, the STZ group exhibited progressive changes that are in accordance with laboratory measurements. These changes were found to be statistically significant, which is consistent with findings reported by Shebelsky et al. in their study.
The selective impact on pancreatic β cells can be attributed to the structural similarity between the STZ molecule and glucose molecule\textsuperscript{12}. This characteristic leads to the internalization of STZ through the use of GLUT-2 transporters that are present inside these cells. Following this, there is a subsequent occurrence of alkanization of cellular DNA, accompanied by the activation of poly-ADP ribose synthetase. This activation leads to a significant reduction in the cellular ATP levels, thereby limiting the synthesis and release of insulin. Consequently, the occurrence of elevated blood glucose levels in rats with diabetes may be attributed to the direct correlation between the permanent deterioration of beta cells inside the islets of Langerhans in the pancreas, caused by STZ, which subsequently leads to insufficient insulin production\textsuperscript{4}.

Glucotoxicity induces alterations that lead to the generation of Advanced Glycation End-products (AGEs), which presents chemically distinct molecules. They can be generated by many mechanisms, acknowledged by different cellular receptors, and classified as either endogenous or exogenous\textsuperscript{13}. Glycosylation reactions often occur by a condensation process involving the carbonyl groups of reducing sugars and the free amine groups present in nucleic acids, proteins, or lipids. The products exhibit stability and irreversibility, and can induce the activation of many signaling pathways associated with inflammation and oxidative stress\textsuperscript{14}.

The adipose tissue evaluation showed that there were no significant variations in the weight of the omental fat of the diabetic animals compared to the control group (Figure 3). In contrast, the retroperitoneal adipose tissue demonstrated a notable reduction in weight in these tissues in diabetic subjects, although no alteration was observed in the control group (Figure 4). The retroperitoneal decrease in diabetic mice might potentially be attributed to diminished insulin production by pancreatic β cells and the development of insulin resistance, which may stimulate lipolysis and hinder lipogenesis\textsuperscript{15}.
Hyperglycemia contributes to the development of insulin resistance by causing a significant reduction in the number of insulin receptors and subsequently impairing insulin binding to these receptors\textsuperscript{16}. Insulin exhibits a well-established mechanism of action characterized by its ability to inhibit lipolysis and promote lipogenesis in both liver and adipose tissues. Lipolysis is initiated by the suppression of the hormone-sensitive lipase enzyme, which is facilitated by the protein kinase A (PKA) pathway. Insulin inhibits this particular pathway by activating a specific cyclic AMP phosphodiesterase (PDE3B), which subsequently reduces cyclic AMP levels in adipocytes. This mechanism leads to a reduction in the breakdown of triacylglycerol into fatty acids and glycerol, resulting in the storage of these substances as body fat\textsuperscript{17}.

In the presence of insulin resistance, the typical regulatory process for lipid synthesis and degradation is compromised, leading to a decrease in adipose tissue. This observation supports the findings of a study conducted by Takada \textit{et al.}, which reported a decrease in adipose tissue thickness in rats with streptozotocin-induced diabetes\textsuperscript{15}.
The examination of the lipid profile of the animals, as determined by the total cholesterol levels, revealed a relation between the control group and the STZ group (Figure 5). Obesity-related metabolic dysfunction is a chronic endocrine disorder that plays a role in the progressive alteration of arterial hypertension and dyslipidemia, hence increasing the risk of cardiovascular illnesses\textsuperscript{18}. The occurrence of dyslipidemia in individuals with diabetes is attributed to enhanced intestinal absorption and synthesis of cholesterol, leading to higher levels of triglycerides and LDL-c, as well as reduced levels of HDL-c\textsuperscript{19}. There is no substantial alteration in the total cholesterol level among those with diabetes in comparison to those without diabetes\textsuperscript{20}.

Figure 5 – Analysis of the lipid profile based on cholesterol levels in control and STZ rats.

The analysis of the triglyceride levels, in both the control and STZ groups (as seen in Figure 6), showed differences between the groups. The activation of intracellular hormone-sensitive lipase, resulting from insulin insufficiency in individuals with diabetes mellitus, leads to an elevation in the release of non-esterified fatty acids (NEFA) from triglyceride deposits in various tissues. Elevated concentrations of NEFA in the bloodstream stimulate the synthesis of triglycerides in the liver. In addition, the typical suppressive impact of insulin on hepatic synthesis and release of very low-density lipoprotein (VLDL) triglycerides is diminished, resulting in the secretion of bigger VLDL particles with higher triglyceride content. Furthermore, the decrease in insulin levels promotes the development of hypertriglyceridemia by inhibiting the use of VLDL\textsuperscript{19}. This observation is consistent with the findings of Nogueira Junior \textit{et al.}, which demonstrated that diabetes affects lipid metabolism in rats, leading to elevated levels of triacylglycerols\textsuperscript{21}.
The blood examination of liver enzymes AST and ALT revealed a substantial elevation in the STZ group compared to the control animals (P<0.001) (Figure 7). The assessment of liver function through the verification of enzyme activity has been well recognized in clinical practice. Elevated levels of these enzymes are indicative of liver damage. During the progression of diabetes, the STZ molecule employs the GLUT-2 transporter, which allows the increase in intracellular glucose. Similar to pancreatic β cells, hepatocytes are likewise impacted by this medication due to the presence of the same transporter. STZ has been found to induce acute liver damage by disrupting ATP synthesis and impairing hepatocyte function. Furthermore, it has been observed that dysregulated lipid metabolism contributes to the development of hepatic steatosis, which can explain the elevation of blood AST and ALT levels.  

Dysregulated lipid metabolism contributes to the development of hepatic steatosis, which can explain the elevation of blood AST and ALT levels.
3.2 HISTOLOGICAL ANALYSIS

3.2.1 Intestinal Histology

The rats exhibited morphological and structural alterations in their small intestine. Previous research has established that individuals with diabetes have alterations in morphology and enzyme activity, which therefore impact the assimilation of carbohydrates, glucose, and lipid metabolism\(^{25}\).

The experimental group treated with STZ exhibited a statistically significant overall increase in the length of duodenal villi, as seen in Figure 8. The size of the villi in the ileum was shown to be larger in the STZ group. In the jejunum, a notable and statistically significant difference in villi length was seen between the STZ group and the control group, with a larger villi length. In relation to the depth of the intestinal crypts (Figure 9), it was observed that in the jejunum, there was a significant increase in comparison to the control group. Conversely, the depth of the crypts in the duodenum and ileum was found to be smaller when compared to the control group, which is in accordance with the findings reported by Pereira et al.\(^{25}\). In a research conducted by Zhao, Yang, and Gregersen, it was observed that there was an increase in both the length of the villi and the depth of the crypts\(^{26}\).

Figure 8 – Analysis of the length of intestinal villi in control and STZ rats.

Source: The author

Figure 9 – Analysis of the depth of intestinal crypts in control and STZ rats.

Source: The author
The etiology of small intestine cell hyperplasia in STZ rats remains incompletely elucidated. It is postulated that insulin deficiency may play a role in this process, impacting the enzymatic activity of the epithelium. In addition, the presence of hyperglycemia and an excess of intracellular glucose might modify the elongation of the villi and the crypt depth in the duodenum and jejunum, as seen in the study conducted by Zoubi, Mayhew, and Sparrow in 1995.27

Furthermore, it is important to take into account the inflammatory condition resulting from peripheral insulin resistance, obesity, and the other consequential impacts of hyperglycemia in individuals with diabetes. Neuropathy and microvascular involvement are two factors that have a direct impact on the gastrointestinal system.28 Dietary modifications, namely those characterized by high fat and low carbohydrate content, were shown to be correlated with a decrease in intestinal mass throughout all sections of the small intestine in comparison to animals fed with conventional diet. The observed modifications illustrate the influence of dietary factors on the stimulation of an intensified inflammatory reaction. This connection between the inflammatory response and the specific dietary composition provided to the animal model has been established by Lipinski et al.29 Recent research conducted by Pereira et al. has revealed a notable elevation in pro-inflammatory cytokines, including IL-1β, within the small intestine of diabetic rats. The production of this particular cytokine is associated with neuroinflammation and is observed immediately following a neuronal injury.30 Furthermore, the study conducted by Pereira et al. and Ferreira et al. revealed the presence of morphological alterations in neurons, along with a decrease in the density of both the myenteric and submucosal plexus.25,31 Additionally, there are studies that establish a correlation between enteroplasticity and a decrease in motility, a phenomenon that is associated with alterations in neural activity.32

3.2.2 Liver Histology

Was observed the presence of early indications of reversible damage, characterized by cellular swelling, as well as the occurrence of localized areas of inflammation in the STZ group. The results are in accordance with Kohl et al. comparable investigation that examined the hepatic tissue of rats following a 4-week administration of STZ.33

In a study conducted by Hsu et al., it was observed that the administration of STZ over a period of 8 to 12 weeks resulted in the occurrence of ballooning of parenchymal hepatocytes, infiltration of inflammatory cells in the lobular region, and the presence of perivenular and pericellular fibrosis.34 These findings are suggestive of the development of non-alcoholic
hepatic steatosis as a result of the induced condition.

The histological modifications in the hepatic tissue become evident with prolonged administration of STZ. It is important to establish a correlation between the histological observations and the biochemical changes (AST and ALT) suggesting a severe damage in liver tissue.

3.2.3 Kidney Histology

The kidney histology study showed the presence of vacuoles in the renal tubules and epithelial cells in the STZ group. The observed phenomenon can be attributed to the growth of mesangial cells, which indicate the initial manifestation of morphological changes in diabetic nephropathy. Cellular vacuolization of the tubules might manifest as an adaptive response to cellular stress, such as the case observed in hyperglycemia. This result can be related to the accumulation of glycogen or the presence of lipid vacuoles inside the subnuclear region.

The cellular structures in which glycogen is stored are referred to as Armanni-Ebstein cells, and they are observed in cases of extreme hyperglycemia. Previous studies have demonstrated the potential of anti-fibrotic and anti-inflammatory medicines in decreasing vacuolar alterations. The study, conducted by Singh et al. showed the disorganized overall structure and vacuolation of the renal tubules, the thickness of the glomerular membrane and the presence of apoptotic markers.

During the process of cell proliferation, there is an initial increase in the deposition of extracellular matrix, accompanied by a low alteration in mesangial cellularity. This results in hypertrophy of mesangial cells, leading to an increase in both the size and weight of the kidney in the diabetic group. The occurrence of glomerular hypertrophy in diabetes can be related to the hemodynamic alterations and local growth factors that are related to the hyperglycemic condition.

The process of non-enzymatic glycosylation of the glomerular basement membrane and plasma proteins has been found to induce an increase in gene expression and the synthesis of type IV collagen, laminin, and fibronectin. Furthermore, the progression of nephropathy and secondary lesions in DM is related to the presence of AGEs and the production of ROS.

3.2.4 Histology of the Retina

The morphometric analysis of the retina in this study revealed that there was no statistically significant variation in retinal thickness between the rat model with diabetes induced by intraperitoneal injection of streptozotocin after 8 weeks and the non-diabetic rat
group, as reported by Lai and Lo\textsuperscript{40}.

Nevertheless, a notable reduction in ganglion cell count was seen in the cohort of rats subjected to an 8-week induction of diabetes mellitus using streptozotocin, as previously evidenced by Lai and Lo in earlier investigations employing this particular diabetic rat model\textsuperscript{40}. The analysis of number of cell nuclei showed no significant difference between the groups, this result suggested that it is necessary more than 8 weeks of experiment to observe this alteration.

4 CONCLUSION

The results of this study showed an animal model that allows the simulation of diabetes complications. The biochemical and histological results demonstrated that the protocol of streptozotocin administration promotes alteration on the main tissues affected by the hyperglicemic state of diabetic patients. This study can be used for future investigations of diabetic mechanisms as well as for the development of treatment for this chronic disease.
REFERENCES


2. Federation ID. IDF diabetes atlas, tenth. International Diabetes. 2021


