Original Article

Hyperbaric Oxygen in a Streptozotocin-Induced Animal Model: Pancreatic Tissue Analysis, Blood Glucose and HbA1c

Titut Harnanik[1,2*], Christian Bambang Sulistio[1], Jakaria Zansen[1], Ludwinna Kezhya Astarini[1]

ABSTRACT

OBJECTIVE: This study aims to examine the effect of hyperbaric oxygen (HBO) on the improvement of blood glucose, hemoglobin A1c (HbA1c) levels, and pancreatic apoptotic cells in a streptozotocin (STZ) -induced animal model.

METHODOLOGY: 27 white rats of the Rattus norvegicus strain Wistar were grouped into three: healthy rats (G0), diabetic rats not treated with HBO (G1), and diabetic rats treated with HBO (G2). A single intraperitoneal (i.p.) dose of 75 mg/kg body weight of STZ was used to create a DM model. HBO was performed in a pressurized chamber at 2.4 ATA by inhaling 98% O2 for three 30-minute breaths, alternating with two 5-minute breaths of normal air, for 10 successive days.

RESULTS: The outcome showed a significant decrease in blood glucose levels ($p = 0.000$, $p < 0.05$) and HbA1c levels ($p = 0.017$, $p < 0.05$) in G2 compared to G1. Improvement in pancreatic tissue damage was demonstrated by the absence of apoptotic cells in G2 compared to G1.

CONCLUSION: HBO can repair pancreatic damage in a mouse model of DM. HBO is expected to be used as an adjunct treatment in diabetic cases.

KEYWORDS: Hyperbaric oxygen, diabetes mellitus, apoptotic cells, blood glucose, HbA1c

INTRODUCTION

Diabetes Mellitus (DM) is a life-threatening disease. High blood glucose levels are a sign of insufficient insulin production or the body's failure to utilize the insulin it produces[1]. Previous research shows that oxidative stress leading to cell apoptosis is a major factor in beta-cell damage in type 1 diabetes mellitus (T1DM)[2]. HbA1c is a biomarker that reflects glucose levels in DM patients and is used to monitor DM[3].

According to the International Diabetes Federation (IDF), this incidence rate is predicted to continue to increase, reaching 643 million (11.3%) in 2030 and 783 million (12.2%) in 2045, with most of this increase occurring in developing countries[1]. Demographic transition, changes in habits and lifestyles and delays in diagnosis resulting in not receiving appropriate treatment result in a much higher risk of complications and thus facing a significantly higher risk of complications[4].

Streptozotocin (STZ) is the most widely used diabetogenic chemical to create mouse models of T1DM and type 2 DM (T2DM)[5]. STZ is a glucosamine-nitrosourea that is toxic to pancreatic β-cells. The STZ model, especially at high doses of STZ, is primarily used to study type 1 DM, as this regimen causes severe, extensive β-cell necrosis with complete loss of pancreatic insulin secretion[6]. STZ induction causes oxidative stress and increased secretion of nitric oxide synthase (NOS), which contributes to the destruction and death of pancreatic islet cells[2].

Hyperbaric Oxygen Therapy (HBO) is a therapeutic approach that uses exposure to concentrated pure oxygen (O2) or 100% O2 at increased atmospheric pressure[6]. This research is expected to demonstrate the benefits of HBO in controlling pancreatic apoptotic cells in DM animal models, providing a basis for further research.

METHODOLOGY

The type of research is "True experimental" using a "Randomized post-test control group design only". Before conducting the research, we conducted an animal ethics feasibility test at the Naval Health Institute Surabaya, East Java, Indonesia, on February 3, 2025. After the ethics test was completed and approved, we proceeded to the next stage of the research. Animal models were created, and blood analysis and histopathology of the experimental animal models were carried out in the hyperbaric laboratory of the Faculty of Medicine, Hang Tuah University, Indonesia. Meanwhile, hyperbaric oxygen therapy was carried out in the hyperbaric laboratory of the Naval Health Institute, Surabaya, Indonesia.

Experimental group

The study included 27 white rats (Rattus norvegicus, Wistar strain). The experimental animals were male, healthy, active, aged 8–12 weeks, and weighing 170–220 grams. The experimental animals were separated into 3 groups: the normal group (Group 0/G0), the

[1]*Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia*
[2]*Naval Health Institute Drs. Med. R. Rijadi S, Phys., Surabaya, Indonesia*
Correspondence: *harnanik.titut@hangtuah.ac.id*
doi: 10.22442/jlumhs.2026.01431
Received: 05-12-2025 *Revised: 04-05-2026*
Accepted: 01-06-2026 *Online First: 27-07-2026*

2026 © This is an Open Access article distributed under the terms of the Creative Commons Attribution – Non-Commercial 4.0 International License, which permits unrestricted use, distribution & reproduction in any medium provided that the original work is cited properly.

experimental group of diabetic animals without HBO therapy (Group 1/G1), and the experimental group of diabetic animals given HBO therapy (Group 2/G2). Each group was placed in a separate cage. G1 and G2 received STZ 75 mg/kg BW i.p. After induction, on the third day, the blood glucose levels of the experimental animals were checked to determine whether they had diabetes.

Hyperbaric Oxygen

Group 2 was given HBO. Group 2 underwent HBO at a continuous depth of 2.4 ATA within 10 minutes. They then proceeded to 98.9% O_2 inhalation for 90 minutes. Administration of 100% O_2 was divided into three sessions and two air-breathing intervals of 5 minutes. The duration of one 100% O_2 inhalation session is 30 minutes. Then, breathe air at a pressure that gradually decreases to normal levels (1 ATA) within 10 minutes. Group 2 received HBO for 10 consecutive days with 50% humidity and a temperature of 28°C.

Blood sampling and collection

Blood was drawn intracardially to measure blood sugar and HbA1c levels. In this method, rats were anaesthetized with ketamine at 0.5 mg/kg body weight of ketamine. Afterwards, the rats were kept anaesthetized for 5 to 10 minutes, until they no longer responded to pain. The rats were then surgically withdrawn, and blood was drawn intracardially using a 3 ml syringe. The rats did not survive after the blood was drawn intracardially.

Blood sugar levels

Blood glucose concentrations were determined via the glucose oxidase biosensor technique. The steps include washing and drying your hands, preparing the equipment and test strip, drawing blood from the syringe onto the strip, and then reading the results on the Gluco Dr. glucometer eleven seconds later. Reference blood sugar levels extend from 80 to 120 mg/dL.

HbA1c levels

Here are the general steps for HbA1c testing in rats. HbA1c levels were measured using the enzymatic fluorescence method. The heart collection procedure is also invasive and is generally used in studies that require larger blood volumes. Rats are anaesthetized, and blood is drawn from the heart with a syringe. Sample Preparation: The collected blood is then processed to separate the red blood cells from the plasma. Often, red blood cells containing hemoglobin are used for HbA1c analysis. HbA1c analysis uses enzymes to measure the level of glucose bound to hemoglobin, which is then used to calculate HbA1c levels. HbA1c measurement results are expressed as a percentage, which reflects the proportion of glycated hemoglobin. The normal range of HbA1c in rats may differ from humans and needs to be determined based on the research protocol used. HbA1c should be reported in NGSP/DCCT units (%).

Pancreas histopathology

Rat pancreas sections were taken for histopathological examination on the 10th day after administration of HBO. Pancreas tissue was fixed in 4% formaldehyde at 4°C for 24 h, and embedded in paraffin wax. The pancreatic tissue was paraffin-embedded, then sectioned at 4–6 μm using a microtome and stained with hematoxylin and eosin (H&E). The slides were then examined using a light microscope.

RESULTS

After data analysis, a significant difference was obtained $p = 0.000$; $p < 0.05$ between the average blood glucose levels taken from the blood of the normal experimental animal group or negative control group (G0), the type 1 DM model experimental animal that was not given HBO or the positive control group (G1) and the type 1 DM model experimental animal group that was given HBO or the treatment group (G2). In the G0 group, the average blood glucose value was 113,333±5.958, which was lower than in the G1 group, which had an average of 308,889±26,689. The Mann-Whitney U test showed a statistically significant difference of $p = 0.000$ ($p < 0.05$) in blood glucose levels between G0 and G1 ($p = 0.000$; $p < 0.05$). In the G1 group, the average blood glucose value was 308.889±26.689, which was higher than in the G2 group, with a mean of 165.111±23.476. The comparison between the G1 group and the G2 group showed a significance of $p = 0.000$ ($p < 0.05$), so that a significant difference was found in the average blood glucose levels in G1 and G2. The average blood glucose levels in the three study groups are shown in **Figure 1**.

In HbA1c levels, data analysis revealed a significant difference in the mean HbA1c levels taken from the blood of normal Wistar rats (G0), untreated type 1 DM (G1), and HBO-treated type 1 DM (G2). In the G0 group, the average HbA1c value was 4.756±0.467, lower than that of the G1 group, which had an average of 8.256±0.713. A post-hoc test using the Tukey (Honest Significant Difference) technique found a significant difference between the G0 and G1 groups ($p = 0.000$; $p < 0.05$). It can be concluded that there was a significant difference in the mean HbA1c levels between the G0 group and the positive control group (G1).

In group G1, namely the experimental animal model of type 1 DM that was not treated with HBO, the HbA1c value was significantly higher $p = 0.017$, $p < 0.05$, with an average of 8.256±0.713, while the treatment group G2 or the experimental animal model of type 1 DM that was treated with HBO, had an average of 7.433±0.543. From these results, there is an effect of administering HBO therapy on HbA1c levels obtained from the blood of STZ-induced animal models. The average HbA1c levels in the three research groups are shown in **Figure 2**.

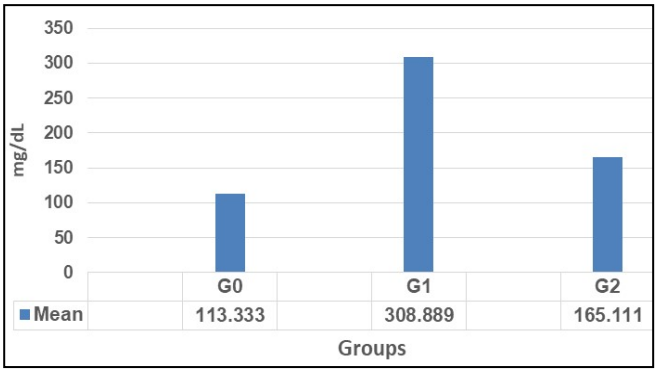

Figure 1: The average blood glucose levels in the three research groups

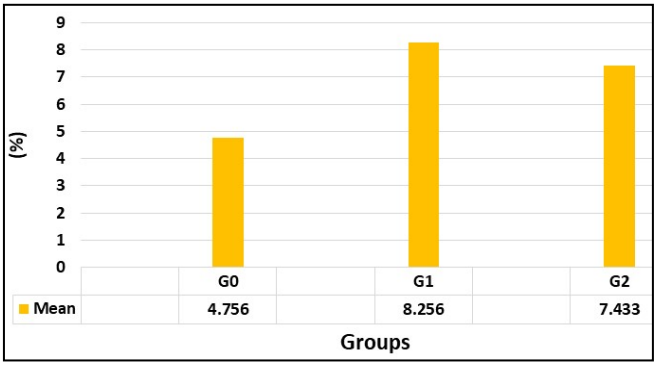

Figure 2: The average HbA1c levels in the three research groups

Histopathology showed a normal pancreatic appearance in group G0. Group G0 shows pancreatic tissue with pancreatic islets indicated by yellow arrows. The islets of Langerhans show a physiological β-cell structure with a normal-sized nucleus in the centre (blue arrow); no apoptotic cells were found. Group G1 shows pancreatic tissue with islets of Langerhans (yellow arrow). In the islets of Langerhans, apoptotic cells show cell shrinkage, chromatin condensation, apoptotic body formation, and phagocytosis. The plasma membrane also shows changes such as blebbing (protrusions in the membrane). Group G2 shows pancreatic tissue with islets of Langerhans (pancreatic islets) indicated by yellow arrows. In the islets of Langerhans, cells have normal structure, with a normal-sized nucleus located in the centre (blue arrow); no apoptotic cells were found. Pancreatic tissue expression in the three research groups is shown in **Figure 3**.

A: Pancreatic tissue in G0. B: Pancreatic tissue in G1. C: Pancreatic tissue in G2. Yellow arrow: Islets of Langerhans. Blue arrow: Normal β cells with normal-sized and centrally located nuclei, no apoptotic cells. Red arrow: Apoptotic cells with cell shrinkage, chromatin condensation, apoptotic body formation and phagocytosis, also experiencing changes in the plasma membrane such as blebbing (protrusions on the membrane).

DISCUSSION

Diabetes Mellitus (DM) is chronic and progressive. Persistently high blood sugar levels are influenced by insulin production and its action[7]. Hyperglycemia causes hypoxia because cells, including mitochondria, increase oxygen consumption. Hyperglycemia and hypoxia are inseparable and mutually influencing conditions, which can lead to excessive endothelin-1 production and the induction of apoptosis[8]. Hyperglycemia has toxic effects that damage nearly all body cells, including the endoplasmic reticulum (ER) and pancreatic β cells, through various mechanisms. Hypoxia causes oxidative stress, which ultimately leads to inflammation, cell damage, and death[9].

Pancreatic β-cell apoptosis also plays an important role in the pathophysiology of Type 2 Diabetes Mellitus (T2DM). It is also influenced by the loss of pancreatic β-cell mass from apoptosis, which impairs insulin secretion. This lack of insulin secretion ultimately leads to chronic hyperglycemia[10]. Apoptosis is induced by hyperglycemia. In vitro apoptosis in isolated islets and insulinoma cell cultures is influenced by the balance of pro-apoptotic Bcl-2 factor proteins such as Bad, Bid, Bik, and Bax and anti-apoptotic Bcl family proteins such as Bcl-2 and Bcl-xL, as well as of caspase-mediated signalling. In the intrinsic pathway of mitochondrial membranes, apoptosis can only occur when the concentration of pro-apoptotic Bcl-2 exceeds the concentration of anti-apoptotic proteins. Fundamental DNA repair enzymes and poly(ADP-ribose) polymerase-1 (PARP1) can regulate β-cell death, replication, and insulin secretion[11].

Hyperbaric oxygen has a remarkable effect on the immune system. HBO can be administered to both healthy and diseased individuals. HBO increases dissolved oxygen in plasma and enhances oxygen

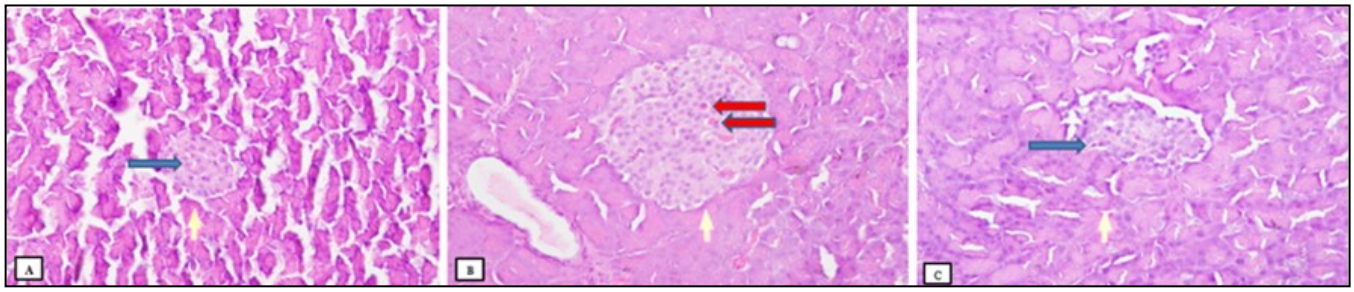

Figure 3: Expression of pancreatic tissue cells

transport to tissues independently of hemoglobin. Furthermore, the resulting increase in barometric pressure can stimulate anti-tumor biological activity through gene expression. This makes HBO superior to other oxygen delivery methods[12]. Oxidative stress damages beta cells. This study intended to determine the impact of HBO on beta-cell function in streptozotocin (STZ)-induced type 1 diabetic rats. By increasing oxygen tension, HBO increases dissolved oxygen in blood plasma, delivering oxygen more effectively to pancreatic tissue, improving the microenvironment, and supporting beta-cell metabolism. HBO helps lower ROS levels, reduces DNA damage, and protects cells from death. HBO reduces inflammation by reducing white blood cell (leukocyte) activation and improving vascular permeability, which helps reduce autoimmune damage to beta cells. Endothelial (blood vessel lining) dysfunction is common in diabetes. HBO improves endothelial function, indirectly supporting blood vessel health in the pancreas and promoting healing and cell function.

HBO significantly reduced the rate of β-cell apoptosis in DM rats through the Bcl-2/caspase-3/pancreatic poly(ADP-ribose) polymerase (PARP) apoptosis pathway. Furthermore, HBO improved pancreatic tissue morphology and increased hepatic glycogen storage in rats. These findings suggest that HBO improves insulin sensitivity, thus lowering blood sugar levels, by reducing the rate of β-cell apoptosis through the Bcl-2/caspase-3/pancreatic PARP apoptosis pathway. Research has focused on reducing blood sugar levels without increasing insulin secretion from the pancreas, but rather on improving insulin effectiveness. This research includes increasing insulin receptor activity and improving insulin sensitivity of upregulating PPAR-γ (peroxisome proliferator-activated receptor-γ)[13].

Excess glucose can covalently bind to haemoglobin irreversibly, a process called glycosylation, or non-enzymatic glycation. Hemoglobin gives blood its bright red color. Glycosylation and protein glycation are distinct processes involving the modification of carbohydrates to proteins. Enzymes mediate glycosylation. During biosynthesis, glycosylation attaches polysaccharides to proteins, affecting protein folding and function. Intracellular glycolysis produces glucose, which further contributes to the formation of AGEs and cell damage.

In contrast, glycation occurs randomly in the intracellular and extracellular spaces, involving the non-enzymatic attachment of glucose to proteins. Glycation ultimately results in the formation of AGEs, which are implicated in diabetes-related diseases. In diabetes, glycation becomes disordered, leading to the accumulation of AGEs, which alter cell structure and function[14]. So, when blood sugar levels increase, more glucose binds to haemoglobin, resulting in a higher HbA1c value[15]. Red blood cells live about 100 to 120 days. Therefore, HbA1c indicates the mean of blood sugar level over the past 60 days[16]. This study shows that HBO can reduce HbA1c levels over 2 months.

CONCLUSION

This study shows that HBO therapy, delivered by inhaling 100% O2 for 3x30 minutes interspersed with 2x5 minutes of rest at a pressure of 2.4 ATA for 10 consecutive days, can reduce apoptosis of pancreatic B cells, thereby reducing blood sugar and HbA1c levels in STZ-induced experimental animals.

Ethical permission: Ethics clearance is granted by the Research Ethics Committee of the Indonesian Naval Health Institute in Surabaya, East Java, Indonesia. No. 02/EC/LKS/II/2025.

Conflict of Interest: No conflicts of interest, as stated by the authors.

Financial Disclosure / Grant Approval: No funding agency was involved in this research.

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Harnanik T: Study design, concept, drafting, data interpretation, literature research
Sulistio CB: Study concept, data analysis
Zansen J: Data interpretation, drafting
Astarini LK: Data collection, questionnaire design, final approval

REFERENCES

1. Hossain J, Al-Mamun, Islam R. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Sci Rep. 2024 March 22; 7(3): e2004.
2. Yavuz O, Dincel GC, Yildirim S, El-Ashram S, Al-Olayan E. Impact of apoptosis and oxidative stress on pancreatic beta cell pathophysiology in streptozotocin-induced Type 1 diabetes mellitus. Cell Tissue and Cell. 2024; 91:
3. Kurniawan LB. HbA1c as Diabetes Mellitus Biomarker and Its Methods. Indonesian J Clin Pathol Med Lab. 30(2): 191-196
4. Lima YMM, Martins FA, Ramalho AA. Epidemiology of Diabetes Mellitus in Adults and Seniors in Rio Branco, Acre, Western Brazilian Amazon. Diabetology. 2024; 5(2): 151-161.
5. Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J. 2023 February 21; 22: 274–294
6. Ortega MA, Fraile-Martinez O, García-Montero C. A General Overview on the Hyperbaric Oxygen Therapy: Applications, Mechanisms and Translational Opportunities. Medicina (Kaunas). 2021 Aug 24; 57(9): 864.
7. Yameny AA. Diabetes Mellitus Overview 2024. J Biosci Appl Res. 2024; 10(3): 641-645.

8. Sada K, Nishikawa T, Kukidome D, Yoshinaga T, Kajihara N, SonodaK, Senokuchi T et al. Hyperglycemia Induces Cellular Hypoxia through Production of Mitochondrial ROS Followed by Suppression of Aquaporin-1. PLoS One. 2016 Jul 6; 11(7): e0158619.
9. Yamagata K, Tsuyama T, Sato Y. Roles of β-Cell Hypoxia in the Progression of Type 2 Diabetes. Int J Mol Sci. 2024 April 10; 25(8): 4186.
10. Weir GC, Gaglia J, Joslin SB. Inadequate β-cell mass is essential for the pathogenesis of type 2 diabetes. Lancet Diabetes Endocrinol. 2020 Jan 29; 8(3): 249–256.
11. Tatsuo Tomita. Apoptosis in pancreatic β-islet cells in Type 2 diabetes. Bosn J Basic Med Sci. 2016 Aug; 16(3): 162–179
12. Wang P, Wang XY, Man CF, Gong DD, Fan Y. Advances in hyperbaric oxygen to promote immunotherapy through modulation of the tumor microenvironment. Front Oncol. 2023 September 15; 13: 1200619.
13. Zhang C, Zhang D, Wang H, Lin Q, Li M, Yuan J et al. Hyperbaric oxygen treatment improves pancreatic β-cell function and hepatic gluconeogenesis in STZ-induced type-2 diabetes mellitus model mice. Mol Med Rep. 2022 January 17; 25(3): 90.
14. Uceda AB, Mariño L, Casasnovas R, Adrover M. An overview on glycation: molecular mechanisms, impact on proteins, pathogenesis, and inhibition. Biophys Rev. 2024 April 12; 16(2): 189–218.
15. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016; 11: 95-104.
16. Gilstrap LG, Chernew ME, Nguyen CA, Alam S, Bai B, McWilliams JM et al. Association Between Clinical Practice Group Adherence to Quality Measures and Adverse Outcomes Among Adult Patients with Diabetes. JAMA Netw Open. 2019 Aug 02; 2(8): e199139.