

Modeling Hyperglycemia Induction Variants In Mice as Preclinical Test Animals

Setiyo Budi Santoso^{1,2*}, Widarika Santi Hapsari^{1,2}, Deksa Yudha Syach Putra¹

¹Department of Pharmacy, Faculty of Health Sciences, Universitas Muhammadiyah Magelang, Indonesia

²Collaboration for Pharmacology and Clinical Pharmacy Studies, Universitas Muhammadiyah Magelang, Indonesia

Abstract

Inducing hyperglycemia in animal models is crucial for preclinical research on antidiabetic drug development, especially when genetically diabetic test animals are unavailable. Various methods have been employed to elevate the glycemic levels in test animals. Despite the common use of alloxan monohydrate, streptozotocin, and dextrose monohydrate to induce hyperglycemia in animal models, their comparative effects on glycemic control and pancreatic histopathology remain insufficiently explored. Specifically, the extent of pancreatic impairment induced by these agents, particularly in stable hyperglycemic conditions. This study aims to evaluate and compare the effectiveness of these three agents in inducing hyperglycemia in mice, focusing on their impact on pancreatic histology, in order to identify the most suitable agent for modeling type 2 diabetes based on glycemic stability and minimal pancreatic impairment. Our protocol involved dividing the mice into six groups of five each with a control group. Mice in Groups A and D were exposed to alloxan monohydrate 0.12 mg/gram body weight, Groups B and E to streptozotocin 0.05 mg/gram body weight, and Groups C and F to dextrose monohydrate 6 mg/gram body weight, inducing hyperglycemia for nine days following a seven-day acclimatization period. Pancreatic histopathological examination included assessments of cytoplasmic vacuolization, fat infiltration, and islet deformation. The study found that alloxan monohydrate was more effective than dextrose monohydrate and streptozotocin in inducing and maintaining hyperglycemic stability in mice. Histopathological analysis showed that dextrose monohydrate and streptozotocin posed a lower risk of pancreatic impairment, while alloxan led to noticeable islet deformation and cytoplasmic vacuolization. Our findings suggest that dextrose monohydrate and streptozotocin are preferable for modeling type 2 diabetes due to their stability and relatively mild effects on pancreatic histology.

Keywords: Animal Models; Alloxan Monohydrate; Dextrose Monohydrate; Streptozotocin; Pancreatic Histopathology

Introduction

Diabetes is now the world's most significant health issue^{1,2}, and is ruled by hyperglycemia^{3,4}. Persistently high glycemic levels may signify an insulin deficiency brought on by the death of pancreatic beta cells, a reduction in glucose receptor sensitivity, or the degradation of tissue receptors^{4,5}, among other serious implications⁶.

Throughout the preclinical stage of developing an anti-diabetic drug, test animals with diabetic pathological characteristics should be readily available^{7,8,9}. Mice are among the alternative study animals employed in laboratories^{10,11,12}, because of their ease of handling¹³, excellent adaptability, and physiological similarities to humans¹⁴. In such studies, diabetic mouse models such as Akita mice, NOD-mice, and Ob-mice are typically employed¹⁵.

To the genetically diabetic test animals is unavailable, alloxan monohydrate and streptozotocin are regularly employed to induce diabetes in mice^{15,16}. Alloxan, a toxic glucose analogue, targets pancreatic beta cells via the GLUT2 transporter, generating reactive oxygen species (ROS) that cause oxidative stress and necrosis, impairing insulin secretion¹⁷. Initially, alloxan induces a transient hypoglycemic phase due to brief insulin stimulation before extensive beta-cell damage occurs^{17,18}. In contrast, streptozotocin alkylates DNA in beta cells, inducing stable diabetes through DNA fragmentation, ROS production, and depletion of NAD⁺ and ATP, leading to irreversible beta-cell damage^{19,20}.

As demonstrated in prior research, alloxan monohydrate induction for one day increased glycemic levels to 127 mg/dL²¹, and induction for three or more days increased it to 164-270 mg/dL^{22, 23}. Meanwhile, three days of streptozotocin induction increased the glycemic level to 137 – 273 mg/dL^{6,24,25},

whereas seven days of dextrose monohydrate induction resulted in a glycemic index of 148 – 155 mg/dL^{8,9}.

Despite the common use of alloxan monohydrate, streptozotocin, and dextrose monohydrate to induce hyperglycemia in animal models, their comparative effects on glycemic control and pancreatic histopathology remain insufficiently explored. Specifically, the extent of pancreatic impairment induced by these agents, particularly in stable hyperglycemic conditions. This study aims to evaluate and compare the effectiveness of these three agents in inducing hyperglycemia in mice, focusing on their impact on pancreatic histology, in order to identify the most suitable agent for modeling type 2 diabetes based on glycemic stability and minimal pancreatic impairment.

Methods

Materials and equipment

Hyperglycemia-inducing preparations included alloxan monohydrate (Sigma-Aldrich, Louis, MO), streptozotocin (Sigma-Aldrich, St. Louis, USA) and 40% dextrose monohydrate (Otsuka, Malang, Indonesia). Metformin (Dexa Medica Palembang, Indonesia) and Na-CMC (Dai-ichi Kogyo Seiyaku, Kyoto, Japan) were used in the intervention preparations. The induction preparation solvent was citrate buffer (pH 4.5). A glucose strip package and glucometer (easytouch) were used to measure the glycemic index. Consumable supporting protocols included handsoons, 1 ml and 5 ml syringes, blood lancets, and aquadests. Supporting instruments for the protocols include analytical scales, beaker glass, mouse cages, markers, mortars, stampers, and oral probes.

Ethical clearance and animal

Under registration number KE/FK/0328/EC/2022 by the Medical and Health Study

Ethics Committee Ethics of Universitas Gadjah Mada's Faculty of Medicine approved the study protocols. Our study involved 36 male mice of the Balb/c strain with a weight range of 25-30 grammes and gestation period of 2-3 months. All mice were obtained from the Pharmacy Laboratory of Gadjah Mada University, Yogyakarta, Indonesia.

Diabetic-Induced Assay and Evaluation

A total of 36 mice were divided into six groups of five primary test animals and one reserve animal. Following a seven-day acclimatisation period¹², all test groups underwent a nine-day hyperglycemia modelling induction period, followed by a five-day intervention period¹¹.

To induce hyperglycemia, alloxan 0.12 mg/gram body weight (BW) was administered intraperitoneally in groups A and D, streptozotocin 0.05 mg/gram BW intraperitoneally in groups B and E, and dextrose monohydrate 6 mg/gram BW orally in groups C and F. The induction period lasted nine days in a row¹². Following hyperglycemia in all subjects, groups A, B, and C were treated with 0.065 g/g BW metformin orally, whereas groups D, E, and F were treated with Na CMC orally (placebo). Indeed, dextrose monohydrate was induced in groups C and F throughout the intervention period¹². At the end of the acclimatization period, the glycemic index of all mice was measured for the baseline study, then again on the ninth day to evaluate the achievement of hyperglycemia modeling, and on the fourteenth day to demonstrate the stability of the glycemic index following metformin intervention¹¹.

Histopathology assessment

For pancreatic histopathology assessment, the mice were sacrificed by neck dislocation, and the pancreas was removed and prepared in 10% formalin for histological examination

(Bio-Optica). Hematoxylin Eosin (HE) staining was used to prepare and interpret the preparations at the Anatomy and Pathology Laboratory, Faculty of Medicine, Gadjah Mada University. The preparations were examined with an Olympus CX33 microscope (magnifications of 40x, 100x, and 400x), shot with a SIGMA Full-HD Microscope Camera, and processed with ToupLite for Windows x64 version 2.1.17118.20200518. Pancreatic histopathological examination features included cytoplasmic vacuolization, fat infiltration, and islet deformation, with impairment scales of 0 (none), 1 (mild), 2 (moderate), and 3 (severe)²⁶.

Results and Discussion

Stability of Glycemic Index Performance

Throughout the nine-day experiment, both groups of alloxan-induced mice demonstrated glycemic indices of 479 mg/dL (Group A) and 363 mg/dL (Group D). In response to the induction of hyperglycemia, Group A, which received a five-day metformin intervention, exhibited a noticeable decrease in glycemic index (333.6 mg/dL). Conversely, Group D, serving as the negative control, exhibited a continued upward trend, reaching a glycemic index of 428 mg/dL (Figure 1).

Similarly, over the nine-day period, both sets of streptozotocin-induced mice showed initial glycemic indices of 181 mg/dL (Group B) and 140 mg/dL (Group E). Following the induction of hyperglycemia, Group B, treated with metformin for five days, displayed a significant reduction in glycemic index to 165 mg/dL. In contrast, Group E, the negative control, exhibited an ongoing elevation, reaching a glycemic index of 245 mg/dL (Figure 2).

In the case of mice induced with dextrose monohydrate, the nine-day period showed initial glycemic indices of 217 mg/dL

(Group C) and 185 mg/dL (Group F). Post-hyperglycemia induction, Group C, undergoing a five-day metformin treatment, demonstrated a notable decrease in glycemic index (176 mg/dL). Meanwhile, Group F, functioning as the negative control, displayed an upward trend, ending at 197 mg/dL (Figure 3).

Based on the main results (Figure 4), the most substantial elevation in glycemic levels throughout the successive induction phase manifested in the following sequence: alloxan (371%), dextrose monohydrate (49%), and streptozotocin (26%). Following the conclusion of the intervention period, the percentage surge in glycemic levels exhibited a reduction across all three models: alloxan (228%), dextrose (21%), and streptozotocin (15%) (Table 1).

The Pancreatic Histopathology Assessment

Based on the histopathological findings, evident damage is observed in pancreatic cells across all three inductions. The escalation in hyperglycemia corresponds to the grading of pancreatic cell impairment. Notably, the control group, devoid of any intervention, exhibited more pronounced damage compared to the groups undergoing metformin intervention (Figure 5).

In the modeling induction accompanied by metformin intervention, the alloxan group exhibited a moderate degree of damage in the parameters of cytoplasmic vacuolation and alterations in islet shape. In contrast, the streptozotocin and dextrose groups demonstrated mild damage in both of these parameters. Notably, there were no indications of lipid infiltration damage in any of the groups subjected to metformin intervention (Table 2).

Conversely, in the modeling induction without metformin intervention (control groups), all three induction model groups

manifested severe damage in the parameters of cytoplasmic vacuolation and changes in islet shape (Table 2). In relation to the parameter of lipid infiltration, only the alloxan modeling group displayed mild damage, while the remaining groups showed no discernible signs of damage (Figure 6).

This study compares the effects of alloxan monohydrate, streptozotocin, and dextrose monohydrate on glycemic stability and pancreatic impairment. Empirical data on hyperglycemia-inducing agents are provided to guide researchers in selecting appropriate agents for modeling type 2 diabetes, considering stability and relative impacts on pancreatic histology.

Our findings identify alloxan monohydrate as the most reliable hyperglycemic agent, showing a sustained 228% increase in glycemic levels. Interestingly, dextrose monohydrate demonstrates a higher stability index (21%) compared to streptozotocin (15%). These results expand on previous studies, which mainly documented glycemic elevation ranges for alloxan (164–270 mg/dL)^{22,23}, streptozotocin (136.8–273.5 mg/dL)^{6,24,25}, and dextrose monohydrate (148.25–155 mg/dL)^{8,9,27}.

A significant new discovery in our study points to the high stability index of alloxan monohydrate following metformin intervention is associated with prolonged pancreatic damage, marked by cytoplasmic vacuolation and structural alterations in islet cells. The destructive action of alloxan monohydrate on the pancreas^{28,29} leads to reduced insulin levels⁴, as it infiltrates beta cells through the GLUT2 transporter, inducing necrosis through reactive oxygen species (ROS) production, generating hydrogen peroxide and hydroxyl radicals^{4,16,30}.

Similar to alloxan monohydrate, streptozotocin targets beta cells via GLUT2 transporter⁴, leading to necrosis³¹, insulin deficiency and hyperglycemia^{30,32}. By alkylating DNA in beta cells, it induces DNA fragmentation, ROS production, and NAD⁺/ATP depletion, resulting in irreversible pancreatic damage^{19,20}. In contrast, dextrose monohydrate exacerbates hyperglycemia³³, which may lead to pancreatic beta cell damage and decreased insulin secretion due to the production of free radicals and reduced hexokinase activity while also compensatory energy sources^{8,9}.

Our histopathological evaluation highlights cellular responses, particularly cytoplasmic vacuolation, islet shape alterations, and lipid infiltration²⁶. The alloxan control group showed the highest vacuolation severity (score 3), indicating major cellular damage³⁴. Additionally, islet shape alterations scored 3, suggesting potential impairment in glucose metabolism^{35,36,37}. Metformin-treated groups showed no lipid infiltration, suggesting a protective effect^{38,39} possibly due to facilitating the regeneration of impaired Langerhans cells^{40,41}. According to our findings, as in the majority of pancreatic cells⁴², necrotic beta cells disrupt insulin production, leading to hyperglycemia³², and occurs alongside the depletion of the islets of Langerhans⁴³.

Conclusion

The study found that alloxan monohydrate was more effective than dextrose monohydrate and streptozotocin in inducing and maintaining hyperglycemic stability in mice. Histopathological analysis showed that dextrose monohydrate and streptozotocin posed a lower risk of pancreatic impairment, while alloxan led to noticeable islet deformation and cytoplasmic vacuolization. Our findings suggest that dextrose monohydrate and streptozotocin are preferable for modeling type 2 diabetes due to their stability and

relatively mild effects on pancreatic histology.

Acknowledgement

This research is part of the institutional vision revitalization study at the Universitas Muhammadiyah Magelang in the field of pharmacology and clinical pharmacy study.

Funding

We express our gratitude to the research grant “RISETMU” for the scientific manuscript from the Muhammadiyah Council of Higher Education, Research, and Development in 2023.

Conflict of Interest

None declared.

References

1. Dyah A. Perwitasari, Setiyo B. Santosa, Imaniar N. Faridah, and Adrian A. Kaptein, ‘Illness Perceptions and Quality of Life in Patients with Diabetes Mellitus Type 2’, *Indones. J. Clin. Pharm.*, vol. 6, no. 3, pp. 190–199, Sep. 2017, doi: 10.15416/ijcp.2017.6.3.190.
2. S. B. Santoso, D. A. Perwitasari, I. N. Faridah, and A. A. Kaptein, ‘Hubungan kualitas hidup dan persepsi pasien tentang penyakit diabetes mellitus tipe 2 dengan komplikasi’, *Pharmaciana*, vol. 7, no. 1, p. 33, May 2017, doi: 10.12928/pharmaciana.v7i1.4699.
3. H. W. Baynest, ‘Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus’, *J. Diabetes Metab.*, vol. 06, no. 05, 2015, doi: 10.4172/2155-6156.1000541.
4. A. King and A. Austin, ‘Chapter 10 - Animal Models of Type 1 and Type 2 Diabetes Mellitus’, *Diabetes Mellit.*, p. 21, 2017.
5. R. N. Fatimah, ‘Diabetes Melitus Tipe 2’, *J. Major.*, vol. 4, pp. 93–101, 2015.
6. N. Apriani, E. Suhartono, and I. Z. Akbar,

- ‘Korelasi Kadar Glukosa Darah dengan Kadar Advanced Oxidation Protein Products (AOPP) Tulang pada Tikus Putih Model Hiperglikemia’, *J. Kesehat. Masy.*, vol. 11, pp. 48–55, Jul. 2011.
7. A. Hasanah, ‘Efek Jus Bawang Bombay (*allium Cepa* Linn.) Terhadap Motilitas Spermatozoa Mencit yang diinduksi Streptozotocin (STZ)’, *Saintika Med.*, vol. 11, no. 2, p. 92, Mar. 2017, doi: 10.22219/sm.v11i2.4203.
 8. I. A. K. Pramushinta, U. Nurhayati, and Sukarjati, ‘Potensi Ekstrak Etanol Daun Sambung Nyawa (*Gynura procumbens*), Biji Mahoni (*Swietenia mahagoni* jacq) serta Kombinasi Kedua Ekstrak sebagai Herbal Anti Diabetik Dengan Hewan Coba Mencit (*Mus musculus* L.)’, *Semin. Nas. Has. Ris. Dan Pengabd.*, pp. 443–449, 2019.
 9. S. D. Santoso and I. Suryanto, ‘Komparasi Efek Pemberian Minyak Jintan Hitam (*nigella Sativa*) dengan Minyak Zaitun (*olea Europea*) terhadap Penurunan Glukosa Darah pada Mencit (*mus Musculus*) Strain Balb/C’, *J. SainHealth*, vol. 1, no. 1, p. 36, May 2017, doi: 10.51804/jsh.v1i1.76.36-42.
 10. R. A. Nugroho, *Mengenal Mencit sebagai Hewan Uji Laboratorium*. Samarinda: Mulawarman University Press, 2018.
 11. D. Y. S. Putra, S. B. Santoso, and H. Lutfiyati, ‘The Weight Performance Stability of Mice on Modeling Obesity-Associated Hyperglycemia Induced by Dextrose Monohydrate’, *Biol. Med. Nat. Prod. Chem.*, vol. 11, no. 2, pp. 169–173, Sep. 2022, doi: 10.14421/biomedich.2022.112.169-173.
 12. S. B. Santoso, W. S. Hapsari, and R. Setyowati, ‘Modeling of Mice as Test Animals for a Preclinical Study of Hypolipidemic Agents’, *J. Farm. Sains Dan Prakt.*, vol. 9, no. 2, pp. 185–192, Aug. 2023, doi: 10.31603/pharmacy.v9i2.8463.
 13. S. J. Glastras et al., ‘Mouse Models of Diabetes, Obesity and Related Kidney Disease’, *PLOS ONE*, vol. 11, no. 8, p. e0162131, Aug. 2016, doi: 10.1371/journal.pone.0162131.
 14. F. M. S. Putri, ‘Urgensi Etika Medis dalam Penanganan Mencit pada Penelitian Farmakologi’, *Urnal Kesehat. Madani Med.*, vol. 9, no. 2, p. 11, 2018.
 15. C. P. D. Kottaisamy, D. S. Raj, V. Prasanth Kumar, and U. Sankaran, ‘Experimental animal models for diabetes and its related complications—a review’, *Lab. Anim. Res.*, vol. 37, no. 1, p. 23, Dec. 2021, doi: 10.1186/s42826-021-00101-4.
 16. A. Al-awar et al., ‘Experimental Diabetes Mellitus in Different Animal Models’, *J. Diabetes Res.*, vol. 2016, pp. 1–12, 2016, doi: 10.1155/2016/9051426.
 17. O. M. Ighodaro, A. M. Adeosun, and O. A. Akinloye, ‘Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies’, *Medicina (Mex.)*, vol. 53, no. 6, pp. 365–374, 2017, doi: 10.1016/j.medic.2018.02.001.
 18. S. Ahmadi, H. Awliaei, M. Haidarizadeh, and J. Rostamzadeh, ‘The Effect of Ethanolic Extract of *Urtica dioica* Leaves on High Levels of Blood Glucose and Gene Expression of Glucose Transporter 2 (Glut2) in Liver of Alloxan-Induced Diabetic Mice’, *Gene Cell Tissue*, vol. 2, no. 3, Jul. 2015, doi: 10.17795/gct-30355.
 19. A. M. T. A. Nahdi, A. John, and H. Raza, ‘Elucidation of Molecular Mechanisms of Streptozotocin Induced Oxidative Stress, Apoptosis, and Mitochondrial Dysfunction in Rin 5F Pancreatic β Cells’, *Oxid. Med. Cell. Longev.*, vol. 2017, no. 1, p. 7054272, Jan. 2017, doi: 10.1155/2017/7054272.
 20. A. Thabet Al-Nahdi, A. John, and H. Raza, ‘Streptozotocin-induced molecular and metabolic targets in pancreatic beta-cell

- toxicity', Hamdan Med. J., vol. 12, no. 2, p. 65, 2019, doi: 10.4103/HMJ.HMJ_54_18.
21. Irdalisa, Safrida, Khairil, Abdullah, and M. Sabri, 'Profil Kadar Glukosa Darah pada Tikus Setelah Penyuntikan Aloksan sebagai Hewan Model Hiperglikemik', J. EduBio Trop., vol. Vol 3, pp. 1–50, Apr. 2015.
 22. R. S. Dewi, L. Rahayu, and I. Atika, 'Efek Penurunan Kadar Glukosa Darah Rebusan Asparagus (*Asparagus officinalis* L.) pada Mencit yang diinduksi Aloksan', J. Ilmu Kefarmasian Indones., vol. 19, pp. 56–61, 2021.
 23. N. Lolok, H. Rahmat, and P. M. Wijayanti, 'Efek Antidiabetes Kombinasi Ekstrak Kulit Bawang Dayak Dan Kulit Bawang Merah Pada Mencit Yang Diinduksi Aloksan', J. Mandala Pharmacon Indones., vol. 5, 2019.
 24. R. Ocktarini, D. H. Prasetyo, and I. Sjarifah, 'Effect of Herbal Extract of Anting-Anting (*acalypha Australis*) on Blood Glucose Level of Balb/C Mice with Induction of Streptozotocin', Biofarmasi J. Nat. Prod. Biochem., vol. 9, no. 1, pp. 12–16, Feb. 2011, doi: 10.13057/biofar/f090103.
 25. Suwanto and R. Rahmawati, 'Aktivitas Hipoglikemik Diet Pakan Ekstrak Biji Labu Kuning (*Cucurbita moschata* Duch) pada Mencit Diabetes Melitus Terpapar Streptozotocin', J. Pharm. Sci. Clin. Res., pp. 39–41, 2019, doi: DOI: 10.20961/jpscr.v4i1.27292.
 26. C. Csonka et al., 'Isolated hypercholesterolemia leads to steatosis in the liver without affecting the pancreas', Lipids Health Dis., vol. 16, no. 1, p. 144, Dec. 2017, doi: 10.1186/s12944-017-0537-z.
 27. C. D. F. Ira and C. I. NHS, 'Sebagai Antihiperglikemia pada Mencit (*Mus musculus*) yang Diinduksi Dextrosa Monohidrat 40%', J. Pharm. Sci. Pharm. Pract., vol. Volume 2, pp. 27–32, 2015.
 28. Akrom, P. D. Harjanti, and T. Armansyah, 'Efek Hipoglikemik Ekstrak Etanol Umbi Ketela Rambat (*ipomoea Batatas* P) (Eeuk R) pada Mencit Swiss yang Diinduksi Aloksan', Pharmacia, vol. 4, pp. 65–76, 2014.
 29. A. Rohilla and S. Ali, 'Alloxan Induced Diabetes: Mechanisms and Effects', vol. 3, p. 5, 2012.
 30. T. Szkudelski, 'The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas', Physiol Res, 2001.
 31. B. L. Furman, 'Streptozotocin-Induced Diabetic Models in Mice and Rats', Curr. Protoc. Pharmacol., vol. 70, no. 1, Sep. 2015, doi: 10.1002/0471141755.ph0547s70.
 32. M. Abdollahi and A. Hosseini, 'Streptozotocin', in Encyclopedia of Toxicology, Elsevier, 2014, pp. 402–404. doi: 10.1016/B978-0-12-386454-3.01170-2.
 33. F. Pasaribu, P. Sitorus, and S. Bahri, 'The Test of Ethanol Extract of Mangosteen Rind (*Garcinia mangostana* L) to Decrease Blood Glucose Level', J. Pharm. Pharmacol., vol. 1, 2012.
 34. I. Esposito et al., 'Guidelines on the histopathology of chronic pancreatitis. Recommendations from the working group for the international consensus guidelines for chronic pancreatitis in collaboration with the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society, and the European Pancreatic Club', Pancreatology, vol. 20, no. 4, pp. 586–593, Jun. 2020, doi: 10.1016/j.pan.2020.04.009.
 35. S. Fukuhara et al., 'New strategy for evaluating pancreatic tissue specimens from endoscopic ultrasound-guided fine needle aspiration and surgery', JGH Open, vol. 5, no. 8, pp. 953–958, Aug. 2021, doi: 10.1002/jgh3.12617.

36. L. Han et al., 'Human umbilical cord mesenchymal stem cells-derived exosomes for treating traumatic pancreatitis in rats', *Stem Cell Res. Ther.*, vol. 13, no. 1, p. 221, Dec. 2022, doi: 10.1186/s13287-022-02893-1.
37. L. Xia et al., 'Impaired autophagy increases susceptibility to endotoxin-induced chronic pancreatitis', *Cell Death Dis.*, vol. 11, no. 10, p. 889, Oct. 2020, doi: 10.1038/s41419-020-03050-3.
38. S. A. Soelistijo, et al., *Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia 2015*. Indonesia: PB. PERKENI, 2015.
39. I. Pernicova and M. Korbonits, 'Metformin: Mode of Action and Clinical Implications for Diabetes and Cancer', *Adv. Online Publ.*, vol. Volume 10, 2014.
40. P. Ningsih et al., 'Histology of hematoxylin-eosin and immunohistochemical diabetes rat pancreas after giving combination of moringa leaves (*Moringa oleifera*) and clove flower (*Syzygium aromaticum*) extracts', *Open Access Maced. J. Med. Sci.*, vol. 9, no. A, pp. 257–262, May 2021, doi: 10.3889/oamjms.2021.5928.
41. S. Hutahaean, S. Ilyas, and S. Rahayu, 'Histological Change of Pancreatic Islands Following Administration of *Saurauia vulcani* Korth Leaves Extract in Alloxan-induced Diabetic Mice', in *Proceedings of the International Conference of Science, Technology, Engineering, Environmental and Ramification Researches*, Medan, Indonesia: SCITEPRESS - Science and Technology Publications, 2018, pp. 1095–1098. doi: 10.5220/0010104010951098.
42. D. S. Longnecker, 'Anatomy and Histology of the Pancreas'. 2021. doi: 10.3998/panc.2021.01.
43. R. Shah, F. Subhan, S. M. Sultan, G. Ali, I. Ullah, and S. Ullah, 'Comparative evaluation of pancreatic histopathology of rats treated with olanzapine, risperidone and streptozocin', *Braz. J. Pharm. Sci.*, vol. 54, no. 3, Nov. 2018, doi: 10.1590/s2175-97902018000317669.

Table 1. Percentage increase in glycemic levels in mice induced with alloxan monohydrate (A), streptozotocin (B), and dextrose monohydrate (C) over nine days, along with subsequent stability after a five-day metformin intervention.

Treatment Group	Induction Variant Glycemic	Index (Percentage Increase)		
		Baseline	Day-9	Day-14
A	Alloxan Monohydrate	102±12 (-)	479±75 (371%)	334±141 (228%)
B	Streptozocotin	144±20 (-)	181±41 (26%)	165±37 (15%)
C	Dekstroza Monohydrat	145±32 (-)	217±40 (49%)	176±13 (21%)

Table 2. Histopathological evaluation of pancreatic tissue parameters in representative test animal treatment groups.

Treatment	Histopathological Evaluation Parameters		
	Cytoplasmic Vacuolation	Islet Shape Changes	Lipid Infiltration
Modeling Induction with Metformin Intervention			
Alloxan Monohydrate (a)	2	2	0
Streptozocotin (b)	1	1	0
Dekstroze Monohydrat (c)	1	1	0
Modeling Induction (Control)			
Alloxan Monohydrate (d)	3	3	1
Streptozocotin (e)	3	3	0
Dekstroze Monohydrat (f)	3	3	0

Value Interpretation: Normal Morphology (0), Mild Impairment (1), Moderate Impairment (2), Severe Impairment (3).

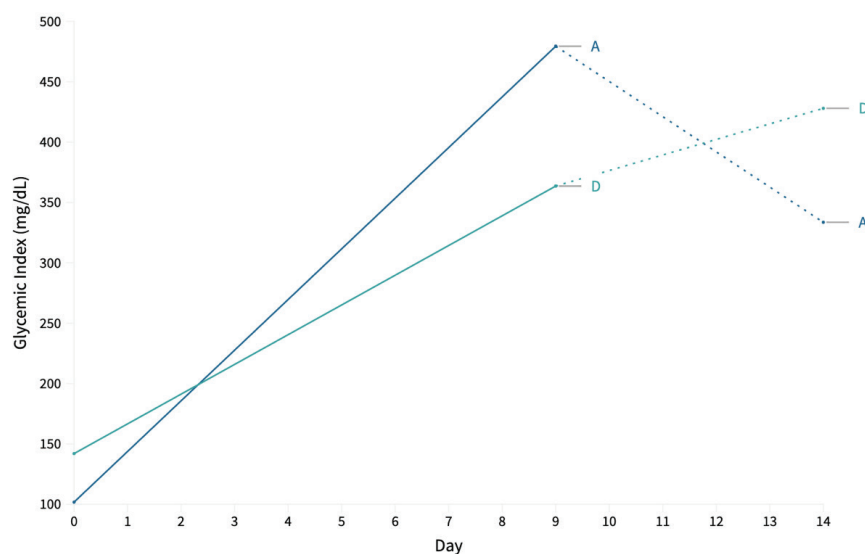


Figure 1. Measurement results of glycemic index in alloxan monohydrate-induced mice within the metformin intervention group (Group A) and the Control Group (Group D).

Solid lines depict the glycemic achievement progression after the induction phase (day-9), and dashed lines represent the stability of glycemic index attainment after the five-day intervention (day-14).

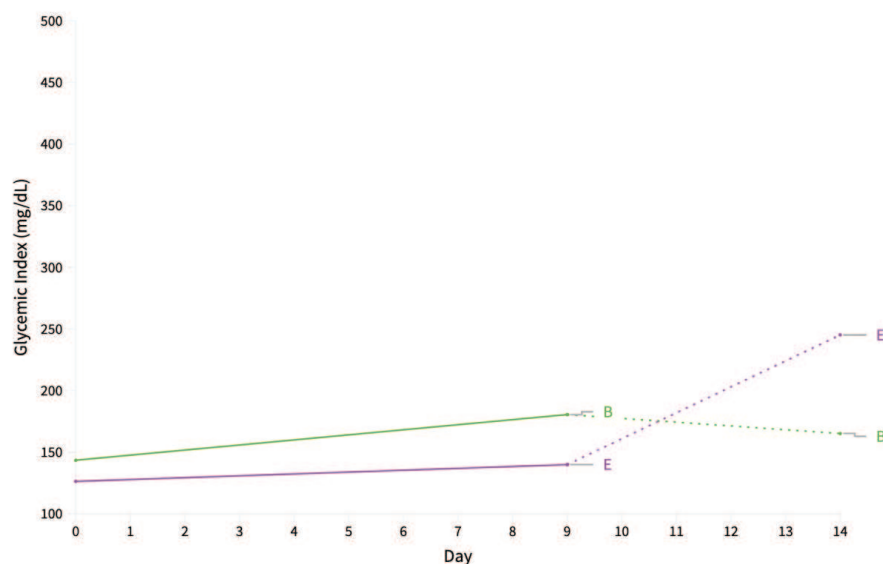


Figure 2. Measurement results of glycemic index in streptozotocin-induced mice within the metformin intervention group (Group B) and the control group (Group E).

Solid lines depict the glycemic achievement progression after the induction phase (day-9), and dashed lines represent the stability of glycemic index attainment after the five-day intervention (day-14).

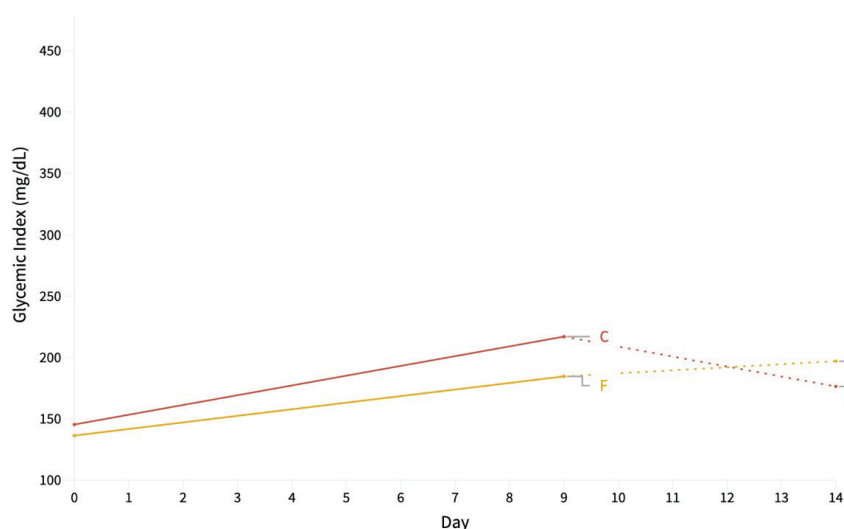


Figure 3. Measurement results of glycemic index in dextrose monohydrate-induced mice within the metformin intervention group (Group C) and the control group (Group F).

Solid lines depict the glycemic achievement progression after the induction phase (day-9), and dashed lines represent the stability of glycemic index attainment after the five-day intervention (day-14).

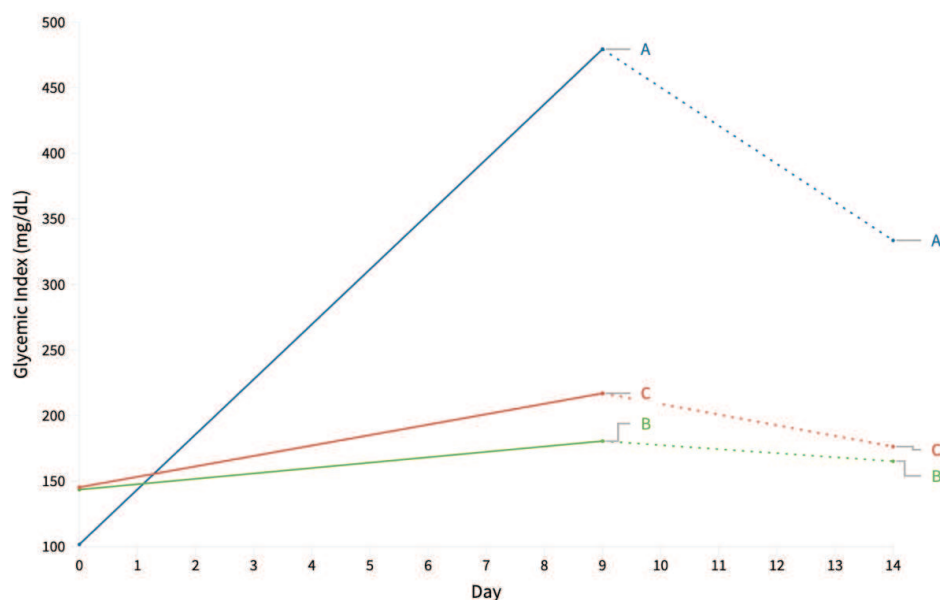


Figure 4. Glycemic index measurements in mice encompassed three distinct induction modalities: alloxan monohydrate (A), streptozotocin (B), and dextrose monohydrate (C).

Continuous lines illustrate the progression of glycemic attainment post the induction phase (day 9), while dashed lines delineate the stability of glycemic index achievement subsequent to the five-day intervention (day 14).

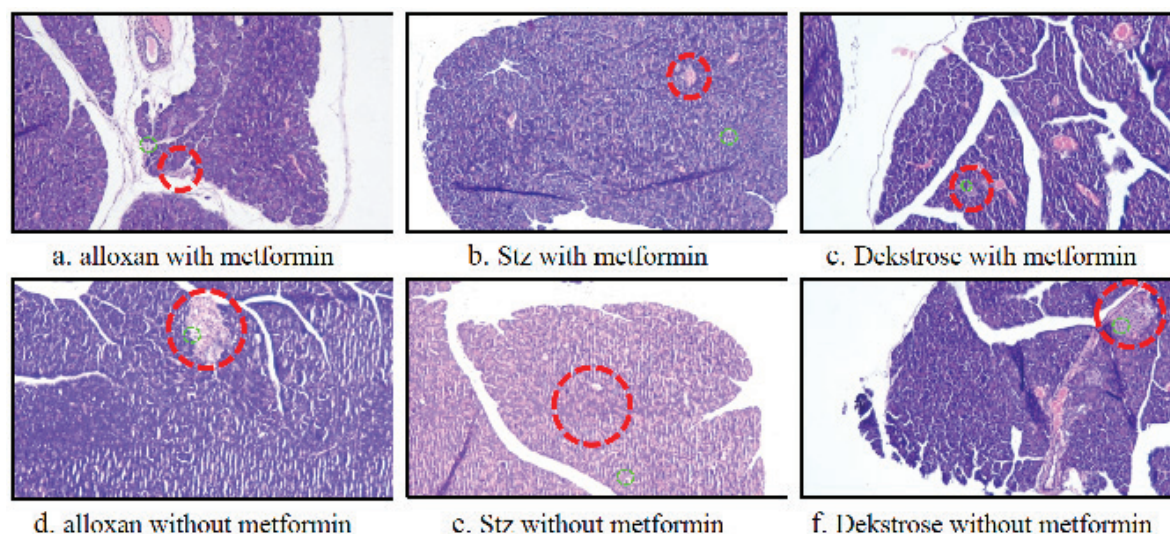


Figure 5. Histopathological examination of pancreatic tissue (100x magnification) in representative samples from six induction variants: alloxan (a and d), streptozotocin (b and e), dextrose (c and f).

Groups a, b, and c underwent metformin intervention, whereas groups d, e, and f were utilized as controls. Islet shape alterations are denoted by red circles, and cytoplasmic vacuolation is indicated by green circles.

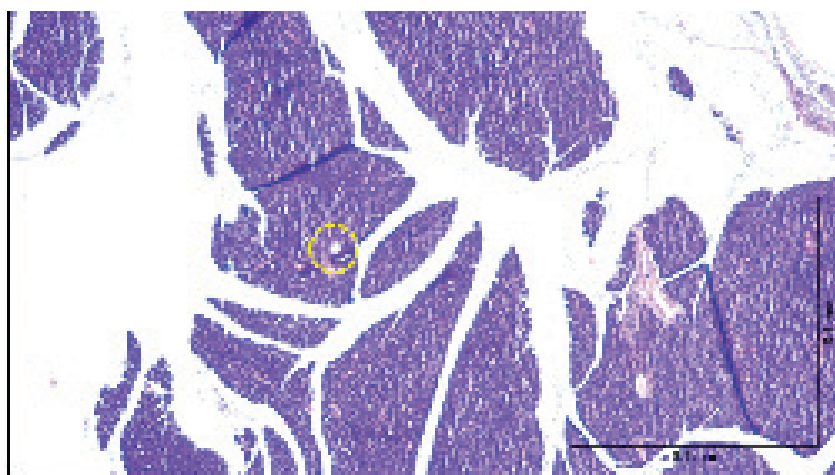


Figure 6. Histopathological examination of pancreatic tissue (40x Magnification) in representative samples of test animals subjected to alloxan induction as the control.

The area of mild lipid infiltration is demarcated by the yellow circle.