Trop J Nat Prod Res, January 2026; 10(1): 6720 - 6724 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Effect of High-Fat and High-Glucose Induction on Histology of the Rat Brain

Shahdevi N. Kurniawan[1]*, Abdullah Abdullah[2], Machlusil Husna[1], Husnul Khotimah[3], Yoga Waranugraha[4]

[1]*Department of Neurology, Faculty of Medicine, Brawijaya University, Malang 65145, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Science, Brawijaya University, Malang 65145, Indonesia*
[3]*Department of Pharmacology, Faculty of Medicine, Brawijaya University, Malang 65145, Indonesia*
[4]*Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia*

ARTICLE INFO

Article history:
Received 24 July 2025
Revised 11 December 2025
Accepted 13 January 2026
Published online 01 February 2026

ABSTRACT

Diabetic neuropathy is a disease caused by nerve damage due to diabetes mellitus. Researches on diabetic neuropathy usually focus on behavioural changes or stimulus-response, and do not assess brain damage. This study aimed to determine the structural and neuronal changes in the brain of diabetic neuropathy rats. Thirty-five Sprague-Dawley rats were divided into four groups. The first three groups were administered high-fat and high-glucose diet for 21 days, followed by a single intraperitoneal injection of streptozotocin (STZ) at 40 mg/kg in the first month (Group 1) and second month (Group 2), and at 20 mg/kg in the third month (Group 3). The fourth group (control) were given sterile water for injection intraperitoneally, followed by high-fat and glucose diet for 10 days only. Fasting blood glucose and body weight of the rats were measured. Thereafter, the rats were sacrificed under ketamine anaesthesia, brain tissues were isolated and used for histological examination and flow cytometric analysis for Nrf2, NF-κB, and Caspase-3. STZ-induction resulted in significant elevation of fasting blood glucose above 7.7 mmol/L accompanied by significant reduction in body weight. Flow cytometric analysis showed no significant changes in the expression levels of Nrf2, NF-κB, and Caspase-3, although, their expression were higher in the diabetic rats, especially in the second month. Histological examination revealed significant brain tissue damage characterized by inflammation, scarring, gliosis, and scrambled tissue architecture in the brain of diabetic rats. These findings provide clear evidence of structural and neuronal changes in the brain of diabetic neuropathy rats.

Keywords: Brain, Diabetic neuropathy, *In vivo*, Streptozotocin.

Introduction

Diabetes mellitus is a common disease and one of the major contributors to global mortality. According to a recent report, an estimated 22,9 million Indonesians are diabetic.[1] Diabetes is a major cause of all deaths resulting from infectious and non-infectious diseases, and diabetic patients are 2-3 times at risk of death compared to normal individuals.[2] Diabetes mellitus is associated with several complications, one of which is nervous system disorders, otherwise called diabetic neuropathy. Diabetic neuropathy can interfere with organ function, and in the peripheral nerves, it reduces motor reflexes and stimuli, and can cause pain. Symptoms range from numbness of the hands and feet, decrease in cognition and memory to brain stem damage. Diabetic neuropathy is more prevalent in type 2 diabetics than in type 1 diabetics.[3]

The pathogenesis of diabetic neuropathy is initiated by high blood sugar levels, which increase the by-products of aerobic metabolism, including increased generation of ROS (Reactive Oxygen Species) such that they overwhelm the body's antioxidant defences. ROS cause disruption of the protein modification process, damage protein structures, damage to nerve cell components, damage to mitochondria, and ultimately leading to nerve cells death.

*Corresponding author. Email: shahdevinandar@ub.ac.id
Tel: +6288803828946

Citation: Shahdevi Nandar Kurniawan, Abdullah Abdullah, Machlusil Husna, Husnul Khotimah, Yoga Waranugraha. The Effect of High-Fat and High-Glucose Induction on Histology of the Rat Brain. Trop J Nat Prod Res. 2026; 10(1): 6720 – 6724 https://doi.org/10.26538/tjnpr/v10i1.41

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The increase in blood glucose also causes the key enzyme of glycolysis, hexokinase, to become saturated, thus triggering the activation of several pathways such as the hexosamine pathway, and the polyol pathway. The pathogenesis of diabetic neuropathy can also be induced by the Receptor of Advance Glycation End Products (RAGE) signalling and the Free-Fatty Acid (FFA) pathway which can increase nerve inflammation. Increased inflammation can also increase the expression of nociceptor proteins in Transient Receptor Potential Cation Channel Ankyrin subtype 1 (TRPA1) and Transient Receptor Potential Cation Channel Vanilloid subtype 1 (TRPV1) that are associated with pain.[3,4]

Research using mice as subjects has been widely conducted worldwide. Modelling using mice has several advantages, which make them a common choice in biomedical research. Some of these advantages include similarity to humans both genetically and physiologically, having a short life cycle and reproduction when compared to other mammals, easy maintenance, and can be genetically modified.[5-7] Several studies have used mice as test animal such as in the test of the interaction between natural compounds and drugs,[5] drug-drug interactions,[7] antioxidant activity tests,[6] food nutrition tests,[8] and new drug tests.[9]

Some diabetes research also uses experimental animals like rats, including research on the effects of pletekan extract on kidney protection in diabetic rats,[10] research on diabetic pregnant rat models,[11] diabetic retinopathy rat model research,[12] and so on. There are also research on diabetic neuropathy, but these focus more on the behavioural response of rats and the morphology of the peripheral nervous system.[13,14] However, in patients with diabetic neuropathy, there are also cognitive, memory, and stimulation processing disorders that indicate damage to the central nervous system, especially the brain, hence, further research regarding structural changes that occur in the central nervous system, especially the brain are needed.[15,16] Therefore, this study aimed to assess the structural and neuronal changes that occur in the brain of rats with diabetic neuropathy, and this will serve as a foundation for developing new treatment approach for diabetic

neuropathy.

Materials and Methods

Materials

The materials used in this study include absolute methanol, Dulbecco′s Phosphate Buffered Saline (Sigma-Aldrich, catalog number D8537-500ML), Fetal Bovine Serum (Gibco, USA, catalog number A3160401), Alexa Fluor® 647 anti-Nrf2 Antibody (Biolegend, USA, catalog number 939206), Phycoerythrin (PE) anti-NF-kB p65 Antibody (Biolegend, USA, catalog number 614154), Per-CP-Cy-7 Anti-Caspase-3 Polyclonal Antibody (BIOSS, USA, catalog number bs-2593R-PerCP-Cy-7), Paraformaldehyde (Pathchem 10% NBF Lot Code:223011-05, BBC Chemical, USA), Flow cytometer (BD FACS Calibur, USA), and CellQuest software (BD Bioscience, USA).

Animals

The experimental animals used in this study were 35 male Sprague-Dawley rats (*Rattus norvegicus*) aged 8-10 weeks with an average weight of 150 - 200 g provided by the Department of Pharmacology, Faculty of Medicine, Brawijaya University, Indonesia. The rats were kept in plastic cages (33 x 22 x 15 cm), where each cage contained 2-3 rats. The rats were allowed access to food and water *ad libitum*. The rats were maintained under controlled experimental conditions (18-26°C temperature, and 40-70%relative humidity). The rats were acclimatized to the laboratory conditions for one week before treatment to allow them adapt to the new environment.

Ethical approval

Ethical clearance for the study was obtained from the Health Ethics Committee, Faculty of Medicine, Brawijaya University ethics committee, with ethical approval number 103 / EC / KEPK / 2024.

Study design

The study is an *in vivo* completely randomized experimental design. The location, temperature, and other environmental conditions were strictly controlled. Thirty-five (35) male Sprague-Dawley rats were divided into four groups consisting of three test groups (10 rats each) and one control group (5 rats). The test groups were high-fat and high-glucose fed rats induced with streptozotocin (STZ). The STZ was administered as a single intraperitoneal injection at a dose of 40 mg/kg body weight in the first and second month (Groups 1 and 2), and at 20 mg/kg body weight in the third month (Group 3). The control (Group 4) consists of healthy rats fed with regular feed, followed by intraperitoneal administration of sterile water for injection. The effect of the treatment was monitored through measurement of body weight, fasting blood sugar levels, and expression of Nrf2, NF-kB, and Caspase-3.

Preparation of streptozotocin (STZ) solution

Streptozotocin (40 mg) was dissolved in 1 mL of sterile injection water in a 1.5 mL microtube to make a 40 mg/mL STZ solution. This concentration falls within the dose range of 35-60 mg/kg body weight which is the ideal dose for diabetes induction in rats.[17,18]

Animal treatment

Prior to STZ induction, the rats were fed with high sugar and fat food (50 g consisting of 60% chicken bran, 30% solid cooking oil and 10% duck egg yolk) and beverage (10% glucose) for 21 days. On the 22nd day following high-fat and glucose intervention, eighteen rats were administered a single intraperitoneal injection of STZ at a dose of 40 mg/kg body weight in the first and second month (Groups 1 and 2) and at 20 mg/kg body weight in the third month (Group 3). The remaining seventeen rats were used as the control (Group 4), and were treated with intraperitoneal injection of sterile injection water only (without high fat and glucose-food or beverage), followed by high-fat and high-glucose diet (glucose: 50 mg/kg body weight, and fat: 30% of total calories) for 10 days.[19]

Measurement of fasting blood glucose and body weight

Fasting blood glucose levels were measured using Easy Touch® blood glucose test strip. Before blood glucose measurement, the rats were fasted overnight (12 hours) without food or high-fat and high-glucose intervention. Blood glucose before and after STZ administration were compared, and the rats with blood glucose levels ≥ 7.7 mmol/L after STZ administration and with stagnant or declining body weight were considered diabetic.[18,20] Rats' body weights were measured using a digital scale before and after diabetes induction.

Animal sacrifice

At the end of the treatment period, six rats each from the normal and diabetic groups were sacrificed under ketamine (300 mg/kg bw, i.p) anaesthesia. Thereafter, the brains were carefully harvested and used for flow cytometric and histological analyses.

Measurement of protein expression

The harvested brain was crushed and washed with 1 mL of PBS (Phosphate-buffered Saline) in a microtube, and centrifuged at 2,500 rpm for 5 min at 10°C. The cells (pellets) were collected and then mixed with 100 µL of cytofix, vortexed, and incubated at 4°C for 20 min. The cells were then added to 500 µL washperm and incubated at 4°Cfor another 10 min. The cells were centrifuged at 2,500 rpm for 5 min at 10°C. The supernatant was discarded and the pellets were supplemented with 100 µL antibody (mouse anti-Nrf2, anti-NF-kB, and Anti-Caspase-3 intracellular antibody). The pellets were washed with antibody dilution buffer (PBS + 10% Fetal Bovine Serum or FBS) and centrifuged. The centrifuged cell pellets were resuspended in 300 µL PBS and transferred to a cuvette for measurement of Nrf2, NF-kB, andCaspase-3 using flow cytometry.[21]

Histopathological investigation

Cross-sections of the brains of rats were stained with Hematoxylin-Eosin (HE) for histopathological analysis. The histological slides were scanned using Aperio Digital Pathology Slide Scanners (Leica Biosystems) at the Laboratory of Pathological Analytics, Faculty of Medicine, Brawijaya University, and analyzed using ImageScope Software to determine histological changes and the level of tissue damage.[22]

Statistical analysis

Data were analyzed using non-parametric tests, including Kruskall-Wallis test for body weight and glucose level, and Mann-Whitney test for flow cytometric analysis, followed by Dunn's post hoc test. Statistical significance was set at $p \leq 0.05$. The statistical analysis was performed using SPSS version 16 for Windows.

Results and Discussion

Blood glucose levels and body weights of rats

Table 1 shows the fasting blood glucose levels and body weights of rats in the normal and diabetic groups. The results show significant ($p < 0.05$) changes in both fasting blood glucose and body weights of the rats before and after STZ-induction. There was inverse relationship between fasting blood glucose and body weight. While fasting blood glucose increased following STZ-induction, body weight decreased. This metabolic data supports classical diabetes phenotypes: Significant increases in blood glucose levels were paralleled by a drop in body weight, a pattern indicative of impaired glucose utilization and muscle catabolism. These metabolic disturbances are also linked with neuropathological outcomes via mechanisms involving Advanced glycation end products (AGEs), Free-fatty acids (FFAs), and oxidative stress pathways.[3,23]

In general, the main characteristic of diabetes mellitus is high fasting blood glucose, accompanied by a rapid weight loss resulting from disturbances in glucose metabolism and dehydration due to more frequent urination.[24]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Blood glucose level and body weight of rats over the treatment period

Category	Condition	Median			Kruskal-Wallis *p-value*
		1st Month	2nd Month	3rd Month	
Blood Glucose (mmol/L)	Normal (n:17)	4.83 ± 0.46	4.97 ± 1.19	4.77 ± 0.49	-
	Diabetic (n = 18)	27.00 ± 0.00*	26.30 ± 1.69*	13.70 ± 7.69*	0.034
Body Weight (g)	Normal (n = 17)	265.33 ± 33.65	276.00 ± 55.75	320.33 ± 28.00	-
	Diabetic (n = 18)	157.33 ± 28.91*	192.67 ± 6.81*	224.33 ± 15.70*	0.039

'*': Significant difference compared to the control group at $p < 0.05$.

Nrf2, NF-kB, and Caspase-3 expressions

The results of the flow cytometric analysis for Nrf2, NF-kB, and Caspase-3 protein expressions are presented in Figure 1. The results revealed a high fluctuation in the protein expressions in the second month in the diabetic neuropathy rat model, although, there were no significant differences in the expression levels of these proteins in both the normal and diabetic rats ($p > 0.05$). Nrf2 and NF-kB are known to act as protective transcription factors in oxidative environments, and their upregulation during the second month may reflect a compensatory cellular response to increasing stress.[25,26] The concurrent increase in Caspase-3 expression also indicates apoptosis of neural cells, especially during the peak induction phase, despite the statistical non-significance. These findings resonate with the dual nature of diabetic stress: chronic inflammation coexisting with compensatory antioxidant activity.[25,27] It is important to state that in the second month of the study, the death of three rats were observed during the STZ induction process, and this may be due to increased stress levels in the brain as indicated by increased levels of Nrf2, NF-kB, and Caspase-3 expressions in the diabetic rats. However, in the third month of the study, there were lower expressions of NF-kB, Nrf2, and Caspase-3 compared to the second month. This could be due to the healing process occurring in the rats, leading to reduction in oxidative stress at the cellular level in the rats' brain (Figure 1). Although, in the third month, there was reduced mortality of the rats, STZ injections at a lower dose (20 mg/kg bw) was required to maintain diabetic state.

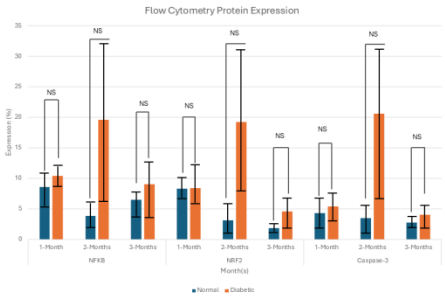

Figure 1: Flow cytometric result showing the expression levels of Nrf2, NF-kB, and Caspase-3 in the brain of normal and diabetic rats. n = 4, NS: not significant ($p > 0.05$)

Histopathological features of the brain of STZ-induced rats

The histological examination revealed significant malformations in the brain tissues of diabetic rats, characterized by increased degrees of gliosis, microgliosis, and neuronal vacuolization from the first to the second month, followed by a mild reduction in the third month (Figure 2). These pathological signs suggest sustained neuroinflammatory processes and glial activation, which are common responses to oxidative and metabolic stress in diabetic neuropathy. This aligns with previous studies indicating that prolonged hyperglycemia contributes to central nervous system damage through vascular dysfunction and neuroinflammation.[27,28] Brain tissue malformation is also marked by the reduction in the number of neuronal cells, indicated by the presence of scars in the brain due to hyperglycaemia-induced stress. The appearance of vascularization in the brain indicates the repair process of the brain in response to damage caused by diabetic neuropathy.[29,30]

Interestingly, despite clear histological pathology, flow cytometric analysis of Nrf2, NF-κB and Caspase-3 expression did not show significant changes across the three months. This could stem from limitations in tissue homogenization, masking region-specific effects of oxidative stress responses.

While the second month of induction (40 mg/kg BW STZ) produced the most pronounced brain tissue damage, it also resulted in higher mortality. This implies a need for model optimization to balance neuropathological severity and animal welfare. Adjustments, such as dose reduction to 20 mg/kg in the third month, succeeded in reducing mortality but may have dampened the observable neuropathological responses.

Overall, this study emphasizes the relevance of including central nervous system assessment in diabetic neuropathy models. Most prior models focused only on peripheral nerves, yet cognitive and structural CNS impairments are increasingly evident in clinical diabetes.[15,16]

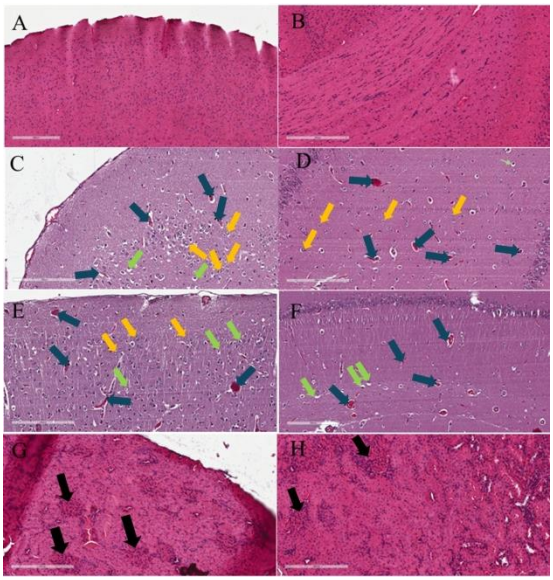

Figure 2: Photomicrographs of brain tissues of normal and diabetic rats; **A:** Cortex region of normal rat brain, **B:** White matter of normal rat brain, **C:** Cortex region of diabetic rats in the 1st month, **D:** White matter of diabetic rats in the 1st month, **E:** Cortex region of diabetic rats in the 2nd month, **F:** White matter of diabetic rats in the 2nd month, **G:** Cortex region of diabetic rats in the 3rd month, **H:** White matter of diabetic rats in the 3rd month.
(Green arrow indicates vacuolization of neuron cell, yellow arrow indicates glial cell activation or gliosis, black arrow indicates microgliosis)

Conclusion

The findings from this study have shown that high-fat and high-glucose diet in conjunction with single intraperitoneal injection of streptozotocin is effective in inducing diabetic neuropathy in

experimental rat model. The structural and neuronal damage to the brain associated with diabetic neuropathy can successfully be confirmed by immunohistochemical analysis of key cellular transcription factors, including Nrf2, NF-kB, and Caspase-3, in addition to histological examination of brain tissues. These findings provide valuable foundation for future investigations exploring the impact of hyperglycemia on brain structure and function.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
The authors express their special thanks to the Faculty of Medicine, Brawijaya University for funding this research.

References

1. Wahidin M, Achadi A, Besral B, Kosen S, Nadjib M, Nurwahyuni A, Ronoatmodjo S, Rahajeng E, Pane M, Kusuma D. Projection of Diabetes Morbidity and Mortality till 2045 in Indonesia Based on Risk Factors and NCD Prevention and Control Programs. Sci Rep. 2024; 14(1):5424. Doi:10.1038/s41598-024-54563-2.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, Regional and Country-level Diabetes Prevalence Estimates for 2021 and Projections for 2045. Diabetes Res Clin Pract. 2021; 183:109119. Doi:10.1016/j.diabres.2021.109119.
3. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, Bril V, Russell JW, Viswanathan V. Diabetic Neuropathy. Nat Rev Dis Primers. 2019; 5(1):41. Doi:10.1038/s41572-019-0097-9.
4. Rachmantoko R, Afif Z, Rahmawati D, Rakhmatiar R, Kurniawan SN. Diabetic Neuropathic Pain. J Pain Vertigo Headache. 2021; 2(1):8–12. Doi:10.21776/ub.jphv.2021.002.01.3.
5. Fayed A, Ramadan HO, Hassan SA, Hussein MA, Roshdy T. Thioredoxin1 Gene Modulates Bcl2/p53/Nf-kb Signaling Pathways in Strawberry Extract/paracetamol-treated Rat Model of Acute Liver Injury. Biomed Pharmacol J. 2022; 15(2):1025–1037. Doi:10.13005/bpj/2438.
6. Hany M, El-Shafai NM, Attia AA, Ibrahim MM, El-Mehasseb IM. *In-vivo* Study on the Rat Heart for Studying the Impact of the Antioxidant Activity of Nanohybrid Composite of Fluoride-based on Chitosan Nanoparticles. Mater Chem Phys. 2022; 290:126610. Doi:10.1016/j.matchemphys.2022.126610.
7. Chen F, Jiang H, Xu J, Wang S, Meng D, Geng P, Dai D, Zhou Q, Zhou Y. In Vitro and In Vivo Rat Model Assessments of the Effects of Vonoprazan on the Pharmacokinetics of Venlafaxine. Drug Des Dev Ther. 2020; 14:4815–4824. Doi:10.2147/DDDT.S276704.
8. Khasanah Y, Ratnayani, Ariani D, Angwar M, Nuraeni T. In Vivo Study on Albumin and Total Protein in White Rat (*Rattus norvegicus*) after Feeding of Enteral Formula from Tempe and Local Food. Procedia Food Sci. 2015; 3:274–279. Doi:10.1016/j.profoo.2015.01.030.
9. Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in Cancer Treatment: from Preclinical Studies to Clinical Practice. Front Pharmacol. 2020; 10:1614. Doi:10.3389/fphar.2019.01614.
10. Roosdiana A, Permata FS, Fitriani RI, Umam K, Safitri A. *Ruellia tuberosa* L. Extract Improves Histopathology and Lowers Malondialdehyde Levels and TNF Alpha Expression in the Kidney of Streptozotocin-induced Diabetic Rats. Vet Med Int. 2020; 2020:8812758. Doi:10.1155/2020/8812758.
11. Chen F, Ge L, Jiang XY, Lai YT, Huang P, Hua J, Lin Y, Lin Y, Jiang X. Construction of the Experimental Rat Model of Gestational Diabetes. PLoS One. 2022; 17(9):e0273703. Doi:10.1371/journal.pone.0273703.
12. Kowluru RA. Retinopathy in A Diet-induced Type 2 Diabetic Rat Model, and Role of Epigenetic Modifications. Diabetes. 2020; 69(4):689–698. Doi:10.2337/db19-1009.
13. Preguiça I, Alves A, Nunes S, Gomes P, Fernandes R, Viana SD, Reis F. Diet-induced Rodent Models of Diabetic Peripheral Neuropathy, Retinopathy and Nephropathy. Nutrients. 2020; 12(1):250. Doi:10.3390/nu12010250.
14. Magadmi R, Alsulaimani MA, Al-Rafiah A, Ahmad MS, Esmat A. Carvedilol Exerts Neuroprotective Effect on Rat Model of Diabetic Neuropathy. Front Pharmacol. 2021; 12:613634. Doi:10.3389/fphar.2021.613634.
15. Zilliox LA, Chadrasekaran K, Kwan JY, Russell JW. Diabetes and Cognitive Impairment. Curr Diab Rep. 2016; 16(9):87. Doi:10.1007/s11892-016-0775-x.
16. Varghese SM, Joy N, John AM, George G, Chandy GM, Benjamin AI. Sweet Memories or Not? A Comparative Study on Cognitive Impairment in Diabetes Mellitus. Front Public Health. 2022; 10:822062. Doi:10.3389/fpubh.2022.822062.
17. Furman BL. Streptozotocin-induced Diabetic Models in Mice and Rats. Curr Protoc. 2021; 1(4):e78. Doi:10.1002/cpz1.78.
18. Ghasemi A and Jeddi S. Streptozotocin As A Tool for Induction of Rat Models of Diabetes: A Practical Guide. EXCLI J. 2023; 22:274–294. Doi:10.17179/excli2022-5720.
19. Gheibi S, Kashfi K, Ghasemi A. A Practical Guide for Induction of Type-2 Diabetes in Rat: Incorporating A High-fat Diet and Streptozotocin. Biomed Pharmacother. 2017; 95:605–613. Doi:10.1016/j.biopha.2017.08.098.
20. Zhang G, Wang X, Ren B, Zhao Q, Zhang F. The Effect of Resveratrol on Blood Glucose and Blood Lipids in Rats with Gestational Diabetes Mellitus. Evid-Based Complement Alternat Med. 2021; 2021:2956795. Doi:10.1155/2021/2956795.
21. Álvarez-Flores MB, Corvinos MS, Santos RG, Valenciano FC. High-sensitivity Flow Cytometry for the Reliable Detection of Measurable Residual Disease in Hematological Malignancies in Clinical Laboratories. Diseases. 2024; 12(12):338. Doi:10.3390/diseases12120338.
22. Ribeiro MC, Bezerra S, Soares AC, Boechat-Ramos R, Carneiro FP, Maciel L, Rosana L, da Silva MV, Viera MP, Monterio IO, Ferreira VM. Hippocampal and Cerebellar Histological Changes and Their Behavioural Repercussions Caused by Brain Ischaemic Hypoxia Experimentally Induced by Sodium Nitrite. Behav Brain Res. 2017; 332:223–232. Doi:10.1016/j.bbr.2017.06.008.
23. Khotimah H, Prima E, Weningtyas AW, Aninditha D, Kalsum U, Kurniawan SN, Rahayu M, Handayani D. Neuroprotective Activity and Antioxidant Effect of *Salacca zalacca* Peel Ethanol Extract on High Glucose Induced Zebrafish (*Danio rerio*) Embryo. Trop J Nat Prod Res. 2021; 5(12):2079–2084. Doi:10.26538/tjnpr/v5i12.7.
24. Li M, Yang X, Sun L, Xiao H, Liu C, Zhang C, Wang Z, Blecker C, Zhang D. Meat Products Consumption and Risk for Alzheimer Dementia and Type 2 Diabetes: A Systematic Review and Meta-analysis Of Cohort Studies. Food Sci Hum Wellness. 2025; 14(1):9250307. Doi:10.26599/FSHW.2024.9250307.
25. Wang R, Liu L, Liu H, Wu K, An J, Wang Q, Liu Y, Bai L, Qi B, Qi B, Zhang L. Nrf2 Protects against Diabetic Dysfunction of Endothelial Progenitor Cells via Regulating Cell Senescence. Int J Mol Med. 2018; 42(3):1327–1340. Doi:10.3892/ijmm.2018.3727.

26. Maguire O, O'Loughlin K, Minderman H. Simultaneous Assessment of NF-κB/p65 Phosphorylation and Nuclear Localization Using Imaging Flow Cytometry. J Immunol Methods. 2015; 423:3–11. Doi:10.1016/j.jim.2015.03.018.
27. Wang Y, Sun H, Zhang J, Xia Z, Chen W. Streptozotocin-induced Diabetic Cardiomyopathy in Rats: Ameliorative Effect of Piperine via Bcl2, Bax/Bcl2, and Caspase-3 Pathways. Biosci Biotechnol Biochem. 2020; 84(12):2533–2544. Doi:10.1080/09168451.2020.1815170.
28. Yang H, Fan S, Song D, Wang Z, Ma S, Li S, Li X, Xu M, Xu M, Wang X. Long-term Streptozotocin-induced Diabetes in Rats Leads to Severe Damage of Brain Blood Vessels and Neurons via Enhanced Oxidative Stress. Mol Med Rep. 2013; 7(2):431–440. Doi:10.3892/mmr.2012.1227.
29. Baptista MGP, Ferreira CGM, Albuquerque YML, D'assunção CG, Alves RC, Wanderley-Teixeira V, Teixeira ÁAC. Histomorphometric and Immunohistochemical Evaluation of the Frontal Cerebral Cortex in Diabetic Rats after Treatment with Melatonin. Pesq Vet Bras. 2020; 40:e06421. Doi:10.1590/1678-5150-PVB-6421.
30. Fikry H, Saleh LA, Abdel Gawad S. Neuroprotective Effects of Curcumin on the Cerebellum in A Rotenone-induced Parkinson's Disease Model. CNS Neurosci Ther. 2022; 28(5):732–748. Doi:10.1111/cns.13805.