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Temporal changes in blood glucose levels and organ histopathology in alloxan-induced diabetic rats

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A B S T R A C T

Background: Diabetes mellitus is a chronic metabolic disorder marked by hyperglycemia due to insulin deficiency or resistance. Alloxan-induced diabetic rat models are widely used to study disease mechanisms and test interventions, yet detailed evaluations of the temporal relationship between hyperglycemia and multi-organ pathology are limited.

Purpose: This study aimed to characterize time-dependent changes in blood glucose and histopathological alterations in the pancreas, liver, and kidneys of alloxan-induced diabetic rats.

Methods: Male Wistar rats were assigned to three groups: control, diabetic for 14 days (A-14), and diabetic for 30 days (A-30). Diabetes was induced with a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Blood glucose and body weight were monitored every 2-3 days. Histological analysis was conducted at the end of each period.

Results: Alloxan induced sustained hyperglycemia (>400 mg/dL), with peak levels of 508.25 mg/dL (A-14) and 544 mg/dL (A-30). A 42.9% dropout rate was recorded, likely due to hypoglycemic episodes shortly after alloxan administration. Diabetic rats showed progressive weight loss and tissue damage. Pancreatic sections revealed islet distortion, cellular degeneration, and vacuolization. The liver exhibited increasing sinusoidal congestion and hepatocyte degeneration, while the kidneys showed glomerular shrinkage, tubular epithelial vacuolization, and peritubular congestion over time.

Conclusion: Alloxan-induced diabetes causes time-dependent hyperglycemia and histopathological damage in the pancreas, liver, and kidneys.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterised by impaired insulin function, either due to the inability to utilise insulin effectively or a complete lack of insulin production, resulting in elevated blood glucose levels (hyperglycemia).[1] In 2022, the global number of adults with diabetes had risen to 828 million, which is an increase of 630 million since 1990.[2] This prevalence increased in most countries, particularly in low- and middle-income regions, while treatment coverage remained relatively low, with 59% of adults aged 30 and older left untreated.[2] Inadequate management of diabetes is associated with serious microvascular and macrovascular complications, including damage to the nervous system, renal Impairment, and retinopathy.[1] Current pharmacological interventions for diabetes mellitus comprise both insulin therapy and a range of oral agents, including α-glucosidase inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, DPP-4 inhibitors, insulin sensitisers and insulin secretagogues.[3]

Animal models are indispensable in diabetes mellitus research, serving as critical platforms for evaluating new therapeutic agents and understanding disease mechanisms. Among these, the alloxan-induced diabetic rat model is widely utilised due to its cost-effectiveness and rapid onset of hyperglycemia.[4] Alloxan targets pancreatic β-cells by generating reactive oxygen species, leading to β-cell death and subsequent insulin deficiency.[5] This model is particularly advantageous for short-term studies focusing on β-cell function and insulin therapy.[4]

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Studies have employed this model to assess the efficacy of various interventions. For example, administration of 150 mg/kg of alloxan to Sprague Dawley rats resulted in a high diabetes induction rate with reduced insulin requirements compared to higher doses, highlighting its suitability for evaluating diabetes treatments.[6] Similarly, the antihyperglycemic effects of aqueous stem bark extracts from *Boswellia dalzielii* were demonstrated in alloxan-induced diabetic Wistar rats, suggesting potential therapeutic applications.[7]

However, the alloxan model has notable limitations. The diabetogenic dose has a narrow therapeutic window, and overdose can lead to significant toxicity in other organs, including the liver and kidneys.[8] The hyperglycemia induced is often transient, with many animals exhibiting spontaneous recovery due to β-cell regeneration, which can confound long-term studies.[9] Alloxan's chemical instability and tendency to induce ketosis can make consistent glycemic control difficult to maintain across experimental cohorts.[9]

Despite the widespread use of the alloxan-induced diabetic model, limited studies have systematically examined the temporal progression of hyperglycemia about organ-specific histopathological changes.[10] Understanding the dynamics of blood glucose fluctuations alongside tissue damage over time is critical for optimising intervention windows and enhancing model reliability. This study aims to address this gap by investigating the temporal changes in blood glucose levels and corresponding histopathological alterations in key organs of alloxan-induced diabetic rats.

METHOD

Study Design

This research was an experimental study with a post-test group design.[11]

Study Site

This study was conducted at the Animal Research Facility, Faculty of Medicine, Universitas Sriwijaya (Palembang, South Sumatra) and Barokah Anatomical Pathology Laboratory (Palembang, South Sumatra).

Materials

The study involved 21 male Wistar rats (3-4 months, 200-300 g). Diabetes was induced using alloxan monohydrate (Sigma-Aldrich, USA), and glucose levels were monitored with a BeneCheck Prime glucometer and strips (General Life Biotechnology, Taiwan). Hematoxylin and eosin staining employed Mayer's hematoxylin, eosin Y (0.5-1% in ethanol), graded ethanol (70-100%), xylene for deparaffinization, and phosphate-buffered saline/distilled water for rinsing. Tissue sections were processed using glass slides, coverslips, staining jars, forceps, and examined under a light microscope. All reagents were of analytical grade.

In Vivo Procedure

Animal Preparation

Twenty-one male Wistar rats (aged 3-4 months) were procured from a certified breeding facility in Palembang, Indonesia. Prior to the study, all animals were clinically assessed and confirmed to be in good health by a licensed veterinarian from the Department of Food Security and Livestock The rats were housed in the Animal Research Facility of the Faculty of Medicine, Universitas Sriwijaya, under controlled environmental conditions (25 ± 2 °C, 12-hour light/dark cycle), with unrestricted access to food and water provided twice daily. A 7-day acclimatization period was observed prior to the commencement of experimental procedures.

Experimental Procedure

Diabetes mellitus was induced via a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). Random blood glucose levels were measured using a BeneCheck Prime glucometer with ~10 µL of blood collected from the tail vein. Rats exhibiting blood glucose levels >200 mg/dL were considered diabetic and included in the study. Animals were randomly assigned to three groups (n=7 per group): (1) normal control (no alloxan injection), (2) diabetic group monitored for 14 days post-alloxan injection (A-14), and (3) diabetic group monitored for 30 days post-alloxan injection (A-30). Body weight and random blood glucose levels were recorded at 10:00 am every 2-3 days: up to day 14 for the control and A-14 groups, and up to day 30 for the A-30 group. At the end of each observation period, rats were euthanized, and the pancreas, liver, and kidneys were collected for histopathological analysis.

Histopathology Assessment

At the end of each observation period, animals were euthanised via chloroform inhalation by ethical standards. The pancreas, liver, and kidneys were collected, fixed in 10% neutral buffered formalin for 24 hours, and processed at the Barokah Anatomical Pathology Laboratory, Palembang. Tissues were paraffin-embedded, sectioned (6 µm), and stained with hematoxylin and eosin. Slides were examined under a light microscope (Olympus CX31) at 400× magnification to assess structural and pathological changes.

Data Analysis

Univariate analysis was conducted by calculating the descriptive statistics such as mean glucose levels for each group at various time points. Histopathological images were qualitatively assessed by a specialized anatomical pathologist, with findings presented as narrative descriptions.

Ethical Consideration

All experimental procedures were approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya (No. 104-2024; 29 May 2024).

RESULTS

Body Weight

After alloxan administration, body weight changes differed across groups (Figure 1). The control group showed a slight, steady weight gain (263–288 g) over 14 days. In contrast, alloxan-treated groups (A-14 and A-30) experienced initial weight loss. The A-14 group lost weight significantly in the first 10 days (230-236 g), with slight recovery by day 14 (238-243 g), while the A-30 group showed a continuous decline (230-248 g) that stabilized at a lower level (238-243 g) after day 10.

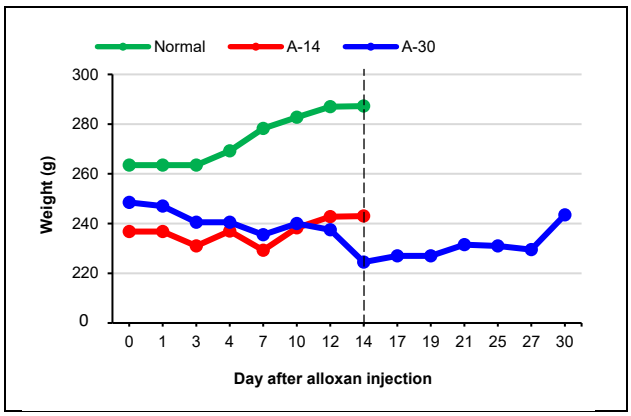

Figure 1. Body weight of rats: Green = controls; Red (A-14) and Blue (A-30) = diabetic rats at 14 and 30 days post-alloxan.

Blood Glucose Levels

Of the 14 alloxan-treated rats, eight developed lethargy and hypoglycemia (35–88 mg/dL) within 24 hours (data not shown). Despite glucose treatment, six rats died by days 2–3, yielding a 42.9% mortality rate. Blood glucose in the A-14 and A-30 groups rose sharply, exceeding 400 mg/dL by day 3, while controls remained normoglycemic (90–133 mg/dL; Figure 2). Peak glucose reached 508.3 mg/dL (A-14) and 544 mg/dL (A-30), with persistent hyperglycemia. The A-14 group displayed minor fluctuations (343–484 mg/dL) but never returned to normoglycemic levels.

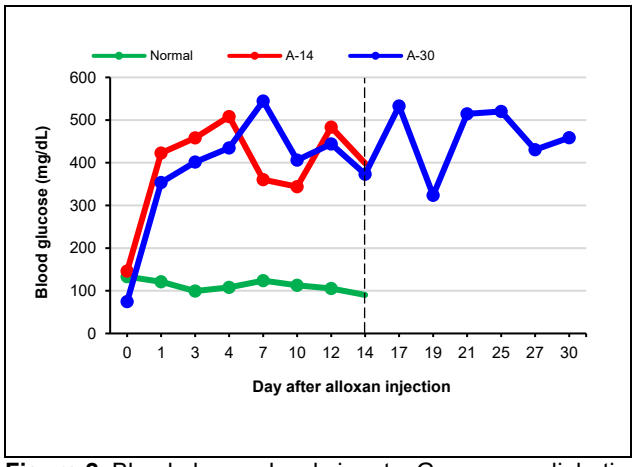

Figure 2. Blood glucose levels in rats. Green: non-diabetic controls; Red (A-14) and Blue (A-30): diabetic rats at 14 and 30 days post-alloxan injection.

Histopathology of Pancreas

Histopathology analysis of pancreas, liver, and kidney tissues showed progressive damage in alloxan-induced diabetic rats. In the pancreas, normal rats (Figure 3A) had well-defined islets of Langerhans. By day 14 post-alloxan injection (Figure 3B), islet borders became irregular, with hydropic changes, vacuolization, and eosinophilic cytoplasm. These alterations were more severe by day 30 (Figure 3C), with further distortion of islet borders, increased vacuolization, and extensive hydropic changes, indicating worsening pancreatic damage.

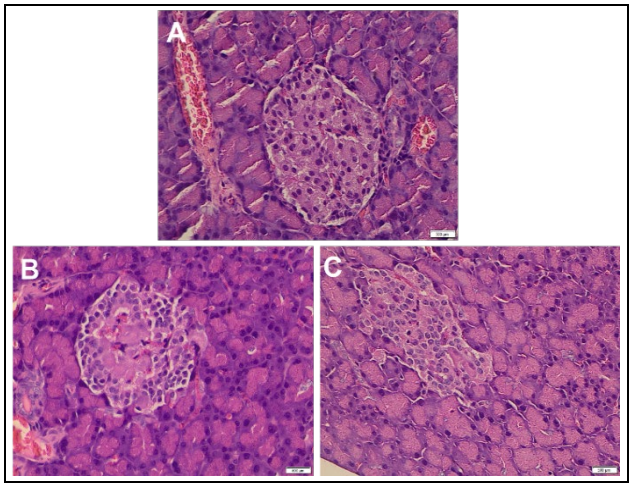

Figure 3. Representative images of the pancreas in (A) normal rats and alloxan-treated rats after (B) 14 days and (C) 30 days of alloxan injection. Scale bar 500 µm.

Histopathology of Liver

In the liver, normal rat hepatocytes (Figure 4A) exhibited typical polygonal morphology and well-defined sinusoidal boundaries. At 14 days post-alloxan injection (Figure 4B), mild sinusoidal dilation and early hydropic changes, including cell ballooning, small vacuoles, and increased eosinophilic cytoplasm, were noted. By day 30 (Figure 4C), severe sinusoidal congestion, pronounced hydropic degeneration, and extensive hepatocyte vacuolization were observed, indicating progressive liver damage.

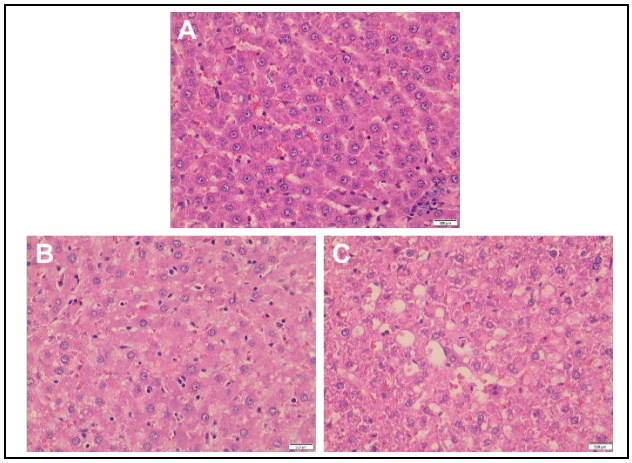

Figure 4. Representative images of the liver in (A) normal rats and alloxan-treated rats after (B) 14 days and (C) 30 days of alloxan injection. Scale bar 500 µm.

Histopathology of Kidney

Renal histology showed time-dependent damage. In normal rats (Figure 5A-B), the kidney maintained normal glomerular and tubular structure. At day 14 post-alloxan injection (Figure 5C-D), mild peritubular capillary congestion and glomerular shrinkage were observed. By day 30 (Figure 5E-F), there was pronounced capillary congestion, eosinophilic changes in the glomeruli, epithelial vacuolization, and hydropic degeneration with cell ballooning, all indicating progressive kidney damage.

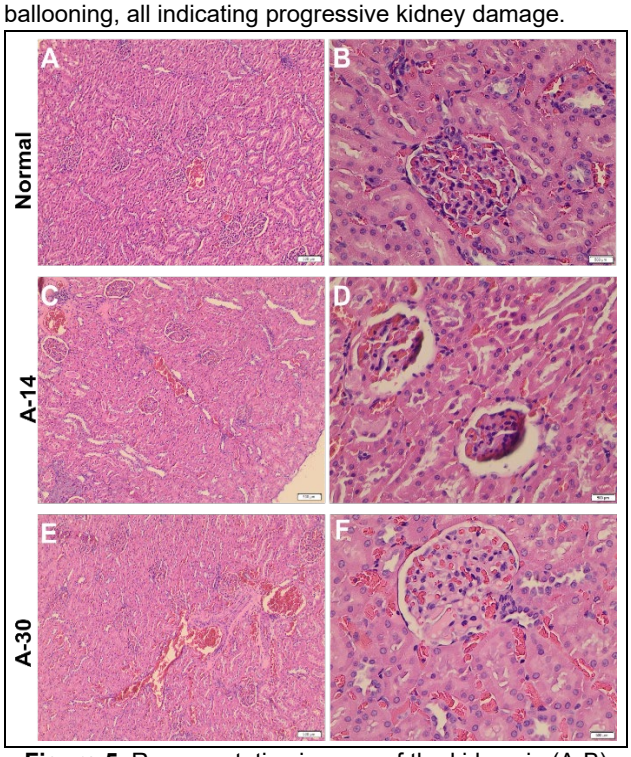

Figure 5. Representative images of the kidney in (A-B) normal rats and alloxan-treated rats after (C-D) 14 days (A-14) and (E-F) 30 days (A-30) of alloxan injection. Scale bar 500 µm

DISCUSSION

The findings of this study demonstrate the successful induction of diabetes mellitus in mice using a single dose of 150 mg/kg alloxan, evidenced by sustained hyperglycemia and weight loss in treated animals. These findings align with the established mechanism of alloxan in selectively destroying pancreatic β-cells through the formation of reactive oxygen species (ROS), leading to insulin deficiency and metabolic dysregulation.[4,12] The more pronounced effects observed in the 30-day group (A-30) compared with the 14-day group (A-14) suggest a progressive and stable diabetic state.[4,13] Although blood glucose levels in the A-14 group exhibited some fluctuation, including a transient decrease around day 7, this variability is characteristic of the alloxan model. It may reflect partial β-cell recovery or individual differences in susceptibility.[14] Importantly, hyperglycemia in all treatment groups remained above the diabetic threshold, confirming the model's reliability in mimicking diabetes-related metabolic changes.

Systemic tissue damage was also observed, particularly in the pancreas, liver, and kidneys, with the severity of the damage increasing over time. This highlights the time-dependent progression of alloxan-induced pathology and supports previous studies showing that hyperglycemia and oxidative stress contribute to multiorgan dysfunction in diabetes models.[15–19] Our observations are consistent with previous reports of progressive metabolic damage and inflammation following alloxan exposure in rodents and other animal models.[17,20]

Alloxan, a glucose analogue with high affinity for the GLUT2 transporter, is selectively taken up by pancreatic β-cells, where it is reduced to dialuric acid.[21,22] This metabolite undergoes autooxidation, generating ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals, which are all potent mediators of irreversible cell death.[13,22] Oxidative stress has emerged as a key driver of β-cell damage and peripheral organ damage. Alloxan has been shown to increase markers of lipid peroxidation (e.g., malondialdehyde) and deplete antioxidant enzymes, including superoxide dismutase, catalase, and glutathione S-transferase, thus creating a redox imbalance that exacerbates tissue injury.[13] These systemic effects underscore the utility of the alloxan model in studying oxidative stress pathways in diabetes. The progression of tissue damage between days 14 and 30 further demonstrates that alloxan-induced diabetes is a dynamic process, involving ongoing metabolic stress and inflammation. This progression, from early hyperglycemia to chronic systemic injury, encompasses multiple phases, including transient hypoglycemia and prolonged oxidative damage.[17,20]

Overall, these findings confirm that alloxan acts as a diabetogenic agent by selectively destroying pancreatic β-cells and inducing sustained hyperglycemia. Progressive histopathological changes in the pancreas, liver, and kidney likely result from a combination of alloxan's direct cytotoxic effects and the secondary consequences of chronic hyperglycemia. Although alloxan is widely used to model type 1 diabetes, its oxidative stress and resulting metabolic disturbances also mimic the pathological features of advanced type 2 diabetes.[12,23,24] This overlap suggests that alloxan-induced models are valuable for investigating β-cell injury and the development of diabetes-related complications across a broader spectrum of the disease.

However, the high early mortality rate (42.9%) observed, particularly within the first 72 hours, is a critical limitation of this study. This mortality is likely due to acute hypoglycemia triggered by the sudden release of insulin following partial β-cell destruction.[14,25] Previous studies have shown that early glucose monitoring and supplementation can significantly reduce mortality in alloxan-induced diabetes models. Compared with streptozotocin, another commonly used diabetogenic agent, alloxan has a narrower therapeutic window and higher acute toxicity, largely due to its rapid ROS production and potential nephrotoxicity.[4,8,10] In contrast, streptozotocin tends to induce diabetes more predictably and with lower mortality. Despite these

challenges, the alloxan-induced diabetes model remains a valuable tool for investigating the pathogenesis of diabetes, particularly oxidative stress, β-cell injury, and therapeutic strategies targeting inflammatory and antioxidant pathways.[27]

CONCLUSIONS AND RECOMMENDATION

This study examined time dependent changes in blood glucose and tissue morphology in alloxan-induced diabetic rats. Progressive histopathological damage in the pancreas, liver, and kidneys was observed, likely due to alloxan's cytotoxicity and sustained hyperglycemia. Precise alloxan dosing, prompt hypoglycemia management, and supportive care are crucial for improving survival and model consistency. Further studies should explore molecular mechanisms of tissue injury and assess targeted therapies, such as antioxidants or anti-inflammatory agents, to reduce multi-organ damage in diabetic models.

REFERENCES

1. Yameny AA. Diabetes Mellitus Overview 2024. *Journal of Bioscience and Applied Research*. 2024;10(3):641-645. https://doi.org/10.21608/jbaar.2024.382794
2. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes prevalence and treatment from 1990 to 2022: a pooled analysis of 1108 population-representative studies with 141 million participants. *Lancet*. 2024;404(10467):2077-2093. https://doi.org/10.1016/S0140-6736(24)02317-1
3. Blahova J, Martiniakova M, Babikova M, Kovacova V, Mondockova V, Omelka R. Pharmaceutical Drugs and Natural Therapeutic Products for the Treatment of Type 2 Diabetes Mellitus. *Pharmaceuticals (Basel)*. 2021;14(8). https://doi.org/10.3390/ph14080806
4. Singh R, Gholipourmalekabadi M, Shafikhani SH. Animal models for type 1 and type 2 diabetes: advantages and limitations. *Front Endocrinol (Lausanne)*. 2024;15:1359685. https://doi.org/10.3389/fendo.2024.1359685
5. Algul S, Ozcelik O. Comprehensive review of animal models in diabetes research using chemical agents. *Lab Anim*. 2025;59(3):356-363. https://doi.org/10.1177/00236772241296199
6. Kim JM. Induction of Diabetes Mellitus Using Alloxan in Sprague Dawley Rats. *Cureus*. 2024;16(6):e63359. https://doi.org/10.7759/cureus.63359
7. Ibrahim AA, Abdussalami MS, Appah J, et al. Evaluation of antihyperglycemic activity of aqueous stem bark extract of Boswellia dalzielii in alloxan-induced diabetic Wistar rats. *Future Journal of Pharmaceutical Sciences*. 2023;9(1):7. https://doi.org/10.1186/s43094-023-00458-4
8. Lucchesi AN, Cassettari LL, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. *J Diabetes Res*. 2015;2015:494578. https://doi.org/10.1155/2015/494578
9. Fajarwati I, Solihin DD, Wresdiyati T, Batubara I. Administration of alloxan and streptozotocin in Sprague Dawley rats and the challenges in producing diabetes model. In: Vol 1174. IOP Publishing; 2023:012035. https://doi.org/10.1088/1755-1315/1174/1/012035
10. Mir MS, Darzi MM, Khan HM, Kamil SA, Sofi AH, Wani SA. Pathomorphological effects of Alloxan induced acute hypoglycaemia in rabbits. *Alexandria journal of medicine*. 2013;49(4):343-353. https://doi.org/10.1016/j.ajme.2013.03.007
11. Bate S, Karp NA. A common control group-optimising the experiment design to maximise sensitivity. *PLoS One*. 2014;9(12):e114872. https://doi.org/10.1371/journal.pone.0114872
12. Singh A, Kukreti R, Saso L, Kukreti S. Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. *Molecules*. 2022;27(3). https://doi.org/10.3390/molecules27030950
13. Ighodaro OM, Adeosun AM, Asejeje FO, Soetan GO, Kassim OO. Time course effects of 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione (alloxan) as a diabetogenic agent in animal model. *Alexandria journal of medicine*. 2018;54(4):705-710. https://doi.org/10.1016/j.ajme.2018.05.005
14. Misra M, Aiman U. Alloxan: an unpredictable drug for diabetes induction? *Indian J Pharmacol*. 2012;44(4):538-539. https://doi.org/10.4103/0253-7613.99348
15. Isirima JC, Uahomo PO. Effect of acalypha wilkesiana on oxidative stress and histopathology of liver and kidney in alloxan-induced diabetic albino rats. Journal of Complementary and Alternative Medical Research. 2023;22(4):11-25. https://doi.org/10.9734/JOCAMR/2023/v22i4463
16. Mir MS, Darzi MM, Khan HM, Kamil SA, Sofi AH, Wani SA. Pathomorphological effects of Alloxan induced acute hypoglycaemia in rabbits. *Alexandria journal of medicine*. 2013;49(4):343-353. https://doi.org/10.1016/j.ajme.2013.03.007
17. Ighodaro OM, Adeosun AM, Asejeje FO, Soetan GO, Kassim OO. Time course effects of 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione (alloxan) as a diabetogenic agent in animal model. *Alexandria journal of medicine*. 2018;54(4):705-710. https://doi.org/10.1016/j.ajme.2018.05.005
18. Misra M, Aiman U. Alloxan: an unpredictable drug for diabetes induction? *Indian J Pharmacol*. 2012;44(4):538-539. https://doi.org/10.4103/0253-7613.99348
19. Queiroz LAD, Assis JB, Guimarães JPT, et al. Endangered Lymphocytes: The Effects of Alloxan and Streptozotocin on Immune Cells in Type 1 Induced Diabetes. *Mediators Inflamm*. 2021;2021:9940009. https://doi.org/10.1155/2021/9940009
20. Han Q, Sun J, Xie W, et al. Repeated Low-Dose Streptozotocin and Alloxan Induced Long-Term and Stable Type 1 Diabetes Model in Beagle Dogs. *Biomed Res Int*. 2022;2022:5422287. https://doi.org/10.1155/2022/5422287
21. Elsner M, Tiedge M, Guldbakke B, Munday R, Lenzen S. Importance of the GLUT2 glucose transporter for

pancreatic beta cell toxicity of alloxan. *Diabetologia*. 2002;45(11):1542-1549. https://doi.org/10.1007/s00125-002-0955-x

22. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. 2008;51(2):216-226. https://doi.org/10.1007/s00125-007-0886-7
23. Attanayake AP, Jayatilaka KAPW, Mudduwa LKB, Pathirana C. β-cell Regenerative Potential of Selected Herbal Extracts in Alloxan Induced Diabetic Rats. *Curr Drug Discov Technol*. 2019;16(3):278-284. https://doi.org/10.2174/1570163815666180418153024
24. Rahman SS, Yasmin N, Rahman A, Zaman A, Rahman MH, Rouf SMA. Evaluation and optimization of effective-dose of alloxan for inducing type-2 diabetes mellitus in long evans rat. *Indian J Pharmaceutical Education and Research*. 2017;51(4):661-666. https://doi.org/10.5530/ijper.51.4s.96
25. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina (Kaunas)*. 2017;53(6):365-374. https://doi.org/10.1016/j.medici.2018.02.001
26. Bacevic M, Rompen E, Radermecker R, Drion P, Lambert F. Practical considerations for reducing mortality rates in alloxan-induced diabetic rabbits. *Heliyon*. 2020;6(6):e04103. https://doi.org/10.1016/j.heliyon.2020.e04103
27. Rosdah AA, Natasha A, Tamzil NS, Subandrate S. Evaluation of Aqueous Extract from Cosmos caudatus Leaves in Alloxan-Induced Diabetic Rats. *Borneo Journal of Pharmacy*. 2025;8(1):107-117. https://doi.org/10.33084/bjop.v8i1.8652.107032