Original Article

Effective Dose Regimen of Streptozotocin for Inducing Diabetes in a Rat Model

Olushola Emmanuel Adeleye[1*], Temtope Ajala[1], Oluwatodimu Adewole Adekoya[2], Adenike Iyabo Adeleye[3]

1. Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria.
2. Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria.
3. Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria.

Use your device to scan and read the article online

How to Cite This Article Adeleye, O. E., Ajala, T., Adekoya, O. A., & Adeleye, O. E. (2024). Effective Dose Regimen of Streptozotocin for Inducing Diabetes in a Rat Model. *Iranian Journal of Veterinary Medicine, 18*(3), 377-386. http://dx.doi.org/10.32598/ijvm.18.3.1005389

http://dx.doi.org/10.32598/ijvm.18.3.1005389

ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by an elevated blood sugar level due to problems with insulin synthesis, effect, or both. Various clinical signs follow DM: Hyperglycemia, polydipsia, polyuria, and polyphagia. Worldwide prevalence is high and predicted to be 592 million by 2035. Animal models are used in the study of diabetes due to ethical issues. Although the streptozotocin (STZ) model is frequently used, it is unreliable due to unexplained acute toxicity and effective dose variability.

Objectives: This research was conducted to determine the effective dose regimen of STZ for inducing diabetes in Wister rats.

Methods: A total of 28 male Wistar rats (160-190 g) were randomly divided into 4 groups (each 7 rats) and monitored for 21 days after diabetes induction with STZ: Control (CTR), diabetics (DIA)1 (60 mg/kg STZ), DIA2 (60 mg/kg STZ twice at 0 and 24 hours), and DIA3 (60 mg/kg STZ thrice at 0, 24 and 48 hours). Plasma glucose was determined with a glucometer. Body weights, feed intake, and fecal output were weighed with a digital balance, while water intake and urine output were measured with a measuring cylinder. Analyses of data obtained were performed using a one-way ANOVA and Tukey's test at a significance level of $P \leq 0.05$.

Results: There was a significant ($P<0.05$) decrease in body weight of the diabetic groups (-15.53%±1.2%, -26.8%±1.2%, -28.5%±1.9%) compared to the CTR (10.5%±2.5%). There was a significant ($P<0.05$) increase in fasting blood glucose concentrations (135.2±9.0, 273.2±6.5, 257.0±5.3 mg/dL) in the people with diabetes compared to the CTR (79.3±1.1 mg/dL). Water intake (56.9±0.9, 72.1±1.7, 77.8±5.5 mL), feed intake (19.4±0.6, 23.3±1.9, 42.1±2.1 g), voided urine (6.34±0.1, 8.39±0.88, 9.8±0.50 mL) and voided feces (10.4±0.26, 11.7±0.43, 8.5±0.17 g) in the diabetic groups increased significantly ($P<0.05$) compared to the CTR (26.5±0.8 mL, 13.4±0.3 g, 1.84±0.08 mL, and 6.5±0.33 g, respectively).

Conclusion: The dose regimen of 60 mg/kg STZ administered intraperitoneally twice (24 hours apart) sustained diabetes for 21 days. We recommend adopting this dose regimen in STZ-induced diabetic studies in male Wistar rats.

Keywords: Dose regimen, Diabetes, Streptozotocin, Wistar rats

Article info:
Received: 11 Nov 2023
Accepted: 30 Feb 2024
Publish: 01 Jul 2024

*** Corresponding Author:**
Olushola Emmanuel Adeleye, Assistant Professor.
Address: *Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria.*
Phone: *+23 (48) 037551972*
E-mail: *adeleyeoe@funaab.edu.ng*

Introduction

As a metabolic disorder, diabetes mellitus (DM) is characterized by an increased level of sugar in the blood (hyperglycemia) resulting from problems with insulin synthesis, effect, or both (ADA, 2014; Thomas et al., 2015). The following criteria are used to confirm the diagnosis of DM: Fasting plasma glucose (PG) concentration ≥126 mg/dL (after ≥8 h of an overnight fast) or PG concentration ≥200 mg/dL 2 hours after ingesting a 75 g oral glucose load after an overnight fast of at least 8 h, or signs of hyperglycemia (such as polyuria, polydipsia, or polyphagia) and a random (non-fasting) PG concentration 200 mg/dL, or hemoglobin A1C (A1C) level ≥6.5% (Blonde et al., 2022). It is necessary to obtain two abnormal test findings from the same sample or two different samples drawn on successive days. However, a glucose reading >200 mg/dL in the presence of DM symptoms confirms the diagnosis (Blonde et al., 2022).

Chronic hyperglycemia, polydipsia, polyuria, polyphagia, impaired vision, unexplained weight loss, lack of energy, diabetic ketoacidosis, hyperosmolar and hyperglycemic non-ketotic syndrome, glycosuria, and weariness are all major clinical consequences of DM (Kumar et al., 2002; ADA, 2014; Rand, 2020).

With variations in frequency among different ethnic groups, there were >425 million cases of DM worldwide in 2017. It is predicted that there will be 629 million cases with type 2 diabetes by 2045, accounting for most (>85%) of the total DM prevalence (Cho et al., 2018; Forouhi & Wareham, 2019).

DM long-term complications include retinopathy, which could lead to blindness; renal disorder, which can lead to kidney failure; peripheral neural disorder, which highly predisposes patients to foot ulcers, limb decapitation, and Charcot's joints; and autonomic neural disorder, which can lead to gastrointestinal, genitourinary, cardiovascular, and sexual dysfunctions. Artery hardening, cardio-vascular, peripheral arterial, and cerebral vascular disorders are more common in diabetic patients. People and animals with diabetes frequently have hypertension and impaired lipoprotein metabolism (Genuth et al., 2003). Diabetes economically drains the global healthcare systems (da Rocha Fernandes et al., 2016).

The two primary classifications for diabetes are type 1 diabetes and type 2 diabetes. Both emerge from complex gene-environment interactions, but they have different pathophysiologies. Type 1 diabetes arises from the immune system destroying the beta-cells in the islets of Langerhans, where insulin is created and secreted. Type 2 diabetes causes hyperglycemia due to pre-existing abnormalities in insulin action and insufficient insulin production (Scheen et al., 2003; Kasuga et al., 2006; Meigs et al., 2009; Blonde et al., 2022).

DM is common in dogs and cats, with hospital incidence rates ranging from 0.4% to 1.2%. When hyperglycemia is severe enough to cause glycosuria, which often happens when blood glucose levels climb (dog: 180 to 220, cats: 220 to 270 mg/dL), clinical signs do not start to show up until that point (Nelson & Reusch, 2014; Rand, 2020). Streptozotocin (STZ; N-nitro derivative of glucosamine) occurs naturally, and it is a broad-spectrum antibiotic that destroys the beta cells of the islets of Langerhans in the pancreas that are responsible for the production of insulin in the body of mammals (Lenzen, 2008; Kintoko et al., 2014). Despite the substantial body of literature on the subject >17000 STZ listings on PubMed reviewers who are unfamiliar with a model of STZ-induced diabetes may find it challenging to plan new investigations accurately. There is no set protocol for the production, dosage, or administration of STZ, and the degree to which STZ induces diabetes can vary greatly (Deeds et al., 2011).

After receiving an intraperitoneal (IP) or intravenous (IV) injection of streptozotocin, experimental diabetic mellitus can be brought on in 2 to 4 days (Wei et al., 2003; Furman, 2021). A dose of 45 mg/kg STZ IP injection induces diabetes in albino Wistar rats (Chao et al., 2018; Eitah et al., 2019). High fat diet (Rosqvist et al., 2014, Schwab et al., 2014; Irannejad et al., 2022), followed a single injection of STZ (45 mg/kg), induces diabetes in rats (Byrne et al., 2015). In addition, 60 mg/kg STZ IP injected once will induce extensive necrosis of Langerhans islets beta cells. Also, a single 65 mg/kg IV injection or two 50 mg/kg IV injections 3 days apart, a single dose at 60 mg/kg IP (Moghtadaei et al., 2021), a single dose at 65 mg/kg IP at 55 mg/kg (Shahsavari et al., 2023), a single dose at 65 mg/kg IV, or 50 mg/kg IV (Szkudelski, 2001; Deeds et al., 2011; Adeleye et al., 2019, Adeleye et al., 2020a, Adeleye et al., 2020b, Cheraghi et al., 2021). Different strains of research animal models react differently to this injection, which should be understandably noted (Mahmoud et al., 2009).

Although there is currently no known cure for the condition, treatment options for type 2 DM include lifestyle changes, managing excessive weight (obesity), oral hypoglycemic agents (OHA), and insulin sensitizers like metformin a biguanide. In contrast, type 1 diabetes is primarily managed through insulin administration (Olokoba et al., 2012; Blonde et al., 2022).

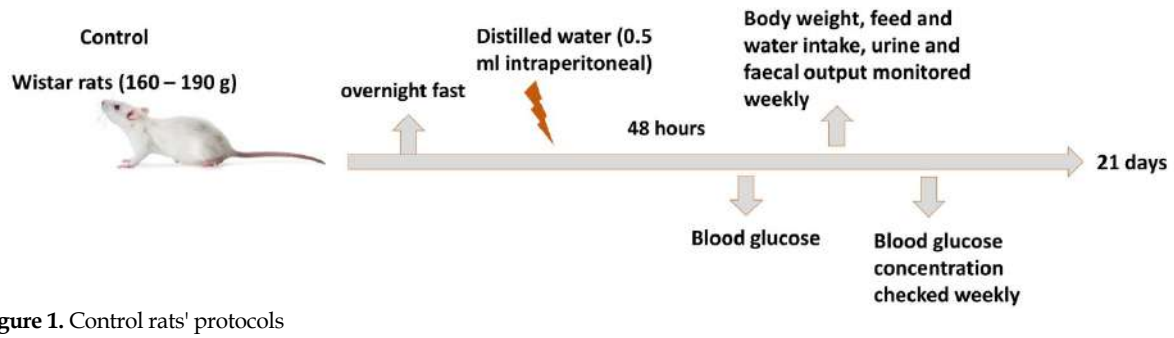

Figure 1. Control rats' protocols

Note: N=7 &, CTR=Control.

Due to concerns about the morality of conducting invasive human research and the numerous uncontrollable factors that could change the uterine environment during clinical studies (Lopez-Soldado & Herrera, 2003), animal models must be used to comprehend the pathophysiology of diabetes better (Rudge, 2013; Baig & Panchal, 2020) and the STZ-induced diabetes model is frequently utilized. The STZ-induced diabetes model, however, has poor dependability because of unexplained acute toxicity and a variable dose schedule. This research was conducted to understand better the right dose regimen, pathophysiological mechanisms, and clinical symptoms in STZ-induced diabetic rats.

Materials and Methods

Animals

Adult male Wistar rats (160–190 g) were housed in well-ventilated standard rat cages in the Experimental Animal Unit of the College of Veterinary Medicine, FUNNAB Veterinary Hospital, Abeokuta, Ogun State, Nigeria, after being obtained from the Teaching and Research Animal House, University of Ibadan. Unless otherwise stated, they were kept in a 12 h light/12 h darkness conditions, fed a conventional rat diet, and always had access to water.

Experimental procedure

These 28 rats were divided into four groups of seven by random selection, and groups diabetics (DIA) 1 through DIA3 were given once daily IP doses of 60 mg/kg STZ (once for DIA1, twice for DIA2, and thrice for DIA3) to induce the diabetic state while group A was not treated. The animals were monitored daily (Figures 1 and 2).

Fasting blood glucose determination

The rats were fasted for 18 hours before their blood samples were taken, but they were given water ad libitum. An Accu-Chek™ glucometer was used to analyze the blood glucose concentration via the glucose oxidase (Nagappa et al., 2003). Briefly, a drop of blood obtained from the tail vein of the rats was placed on the test strip that had already been inserted into the glucometer after it was turned on. The result was viewed from the screen and recorded. This method involves the use of enzymes.

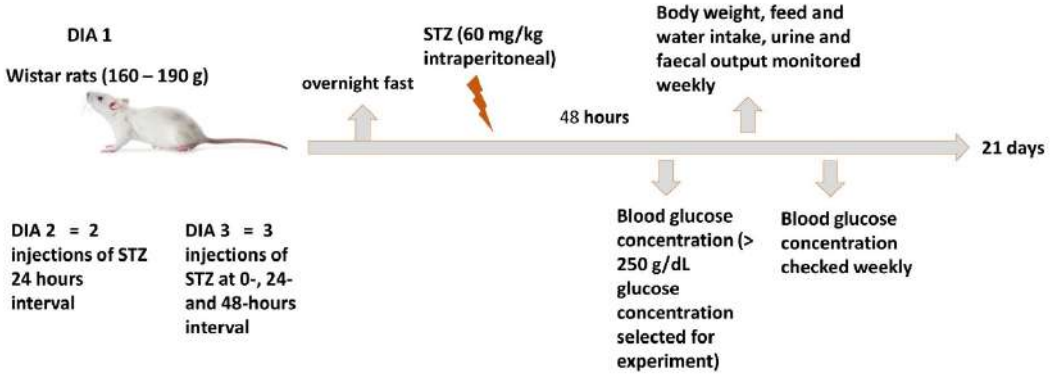

Figure 2. Test rats' protocols

Note: N=7, DIA1=60 mg/kg STZ once, DIA2=60 mg/kg STZ two days consecutively, DIA3=60 mg/kg STZ three days consecutively.

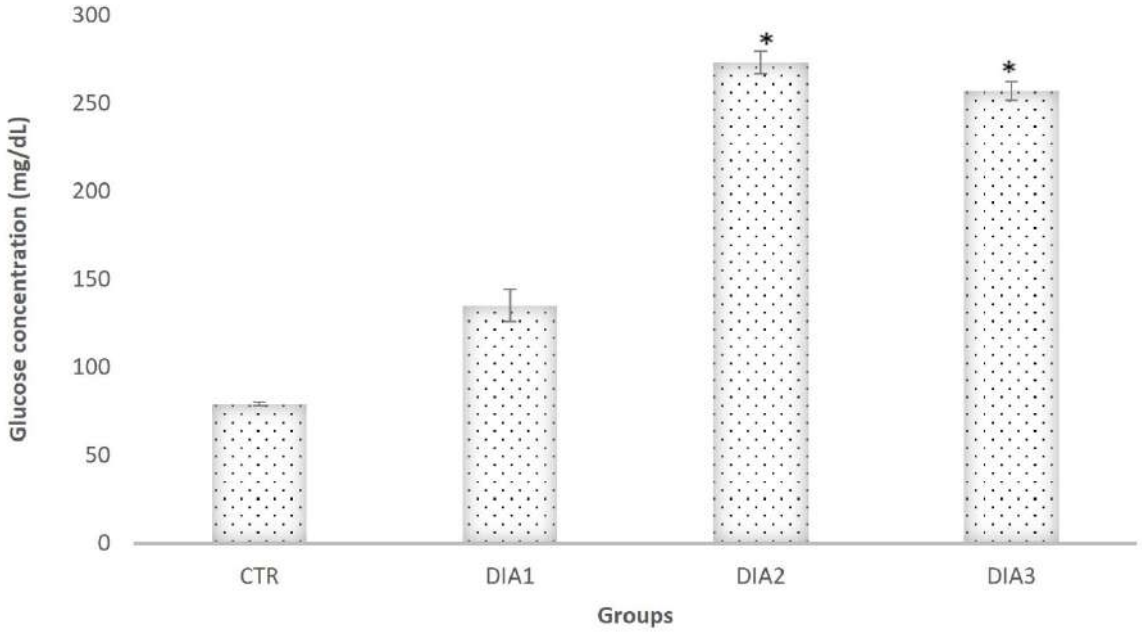

Figure 3. Fasting blood glucose level of control and test rats in mg/dL

Note: N=7, CTR=Control, DIA1=60 mg/kg STZ once, DIA2=60 mg/kg STZ two days consecutively, DIA3=60 mg/kg STZ three days consecutively. *P<0.005 from CTR.

The test strips are loaded with enzymes that react with the blood glucose. There is a color change due to the reaction that the meter measures and translates into glucose concentration in mg/dL. Blood glucose values for each rat were repeated thrice, and the average reading was used. This measurement was performed every other day during the experiment.

Body weight

Throughout the trial, the rats' body weights were checked every three days with a digital weighing scale (Camry®). Percentage weight gain was calculated after the trial. The starting weight was subtracted from the end weight, and the difference was divided by the end weight and finally multiplied by 100 (Equation 1):

1. *Gain or loss (%)=Initial(Gain or loss/Previous value)×100*

Water and feed intake, urine volume, and fecal output

Using a metabolic cage, the daily water (mL) and feed intake (g), with the volume of urine (mL) and feces voided (g), by each rat was measured daily after the initial first week of the experiment. A measuring cylinder was used to measure the water intake and urine output, while a digital weighing balance was used to calculate the feed intake and fecal output (Camry®).

Data analysis

The study data were collected, tabulated, presented in appropriate statistical data form, and expressed as Mean±SEM. Data were analyzed using a one-way analysis of variance (ANOVA), and Tukey's multiple comparison test was performed to compare means. SigmaPlot® software, version 14.5 was used for all analyses, and P<0.05 were considered significant.

Result

Fasting blood glucose level

The fasting blood glucose concentrations of the test and control rats at the end of the experiment are shown in Figure 3. There was a significant increase (P<0.05) in the fasting blood glucose of the test group (DIA1, DIA2, and DIA3) compared to the control group.

Body weight

Control rats gained weight steadily during the experiment (10.5%±2.5%). However, the STZ-treated groups displayed a significant reduction (P<0.05) in body weight (-15.53%±1.2%, -26.8%±1.2%, -28.5%±1.9%) throughout the experimental period (Figure 4).

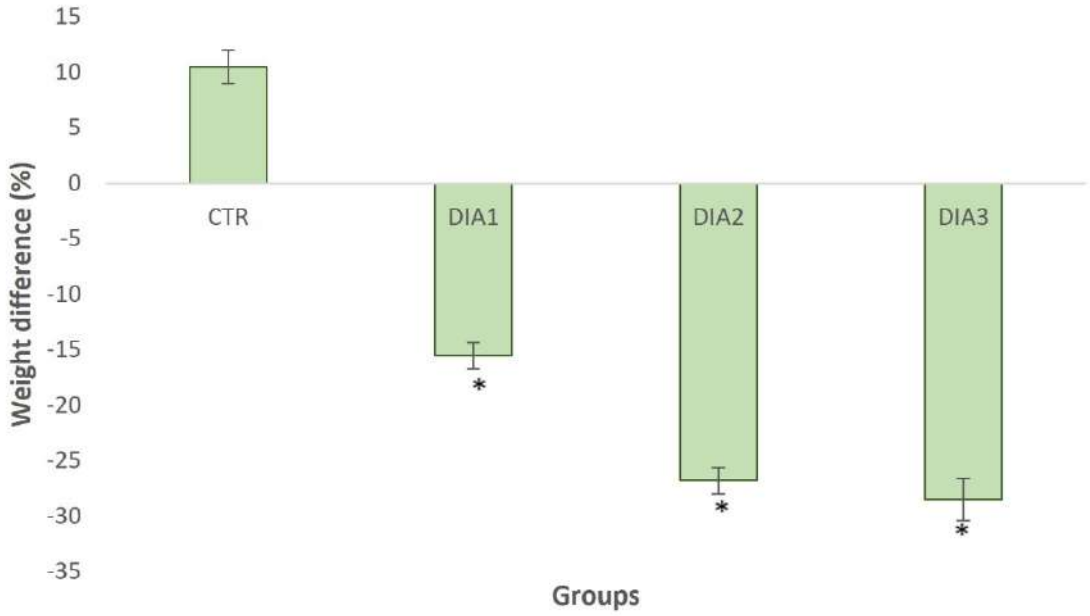

Figure 4. Weight difference of control and test rats in mg/dL

Note: N=7, CTR=Control, DIA1=60 mg/kg STZ once, DIA2=60 mg/kg STZ two days consecutively, DIA3=60 mg/kg STZ three days consecutively. *P<0.005 from CTR.

Feed intake

The intake of food by the STZ-treated groups (19.4±0.6, 23.3±1.9, 42.1±2.1 g) was significantly (P<0.05) greater than that of the controls (13.4±0.3 g) (Figure 5) but there was no significant change in the voided feces between the test groups and the controls.

Intake and output of fluid

The control rats' fluid intake and measured output remained constant throughout the experiment, with intake consistently exceeding output (Figure 6). There was a significant (P<0.05) increase in water intake and urine output in the test groups compared with the controls at the end of the experiment (Figure 6).

Discussion

The result of this research showed that rats that received two successive administrations of streptozotocin 60 mg/kg IP (DIA2) had a higher blood glucose level compared to the rats that received a single dose (DIA1) and three successive doses of streptozotocin IP (DIA3). This result contradicts the findings of Alina et al. (2015), who did not record any significant difference between the blood glucose levels of rats administered one dose versus two consecutive doses of STZ. Hyperglycemia occurs due to the excessive rates of endogenous glucose production and insufficient or lack of insulin in the body (Basu et al., 2004; Robert, 2010).

Weight loss in diabetes reflects the relative loss of the anabolic actions of insulin (Holt et al., 2010). Previous studies have shown that diabetes is accompanied by weight loss (Akbarzadeh et al., 2007; Holt et al., 2010; Gundala et al., 2018; Wang et al., 2022), and this is in tandem with the result of this present study where we report that there was significant weight loss in the diabetic rats compared to the control. This weight loss could also be attributed to the negative fluid balance between fluid intake and output, where there was a significant (P<0.05) decrease in urine output compared to water intake (Figure 6); hence, the animals were in negative fluid balance. Also, compared to the controls, the test rats' urine output was significantly increased, equating to a loss of necessary electrolytes from the body. The increased water intake reduces the concentration or molarity of body electrolytes. This could also lead to the weight loss seen.

Weight change is caused by a long-lasting imbalance of food intake and energy expenditure or negative energy balance (Brown et al., 2019), and weight loss could be explained by reduced energy intake (Svane et al., 2016), which was not the case in this experiment. The food intake of the test rats increased significantly compared to the control rats, and the increase was proportional to the increased dose of STZ. During moments of negative en-

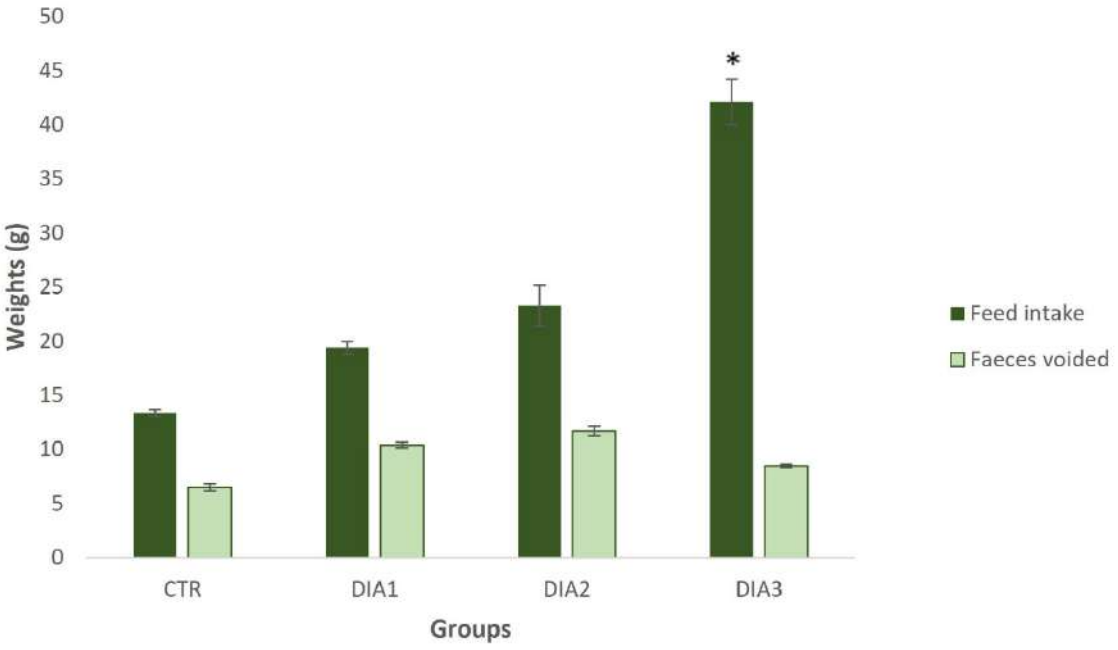

Figure 5. Feed intake and voided faeces of control and test rats in mg/dL

Note: N=7, CTR=Control, DIA1=60 mg/kg STZ once, DIA2=60 mg/kg STZ two days consecutively, DIA3=60 mg/kg STZ three days consecutively. *P<0.005 from CTR.

ergy balance and weight loss, the body attempts to maintain homeostasis through hormonal signaling (e.g. leptin and insulin) and other afferent neuronal signals relaying information to the hypothalamus to stimulate appetite and promote weight gain (Brown et al., 2019). Still, in diabetes, there is a loss of insulin function, which has negative feedback on this system. This would have also contributed to the loss of body weight described above.

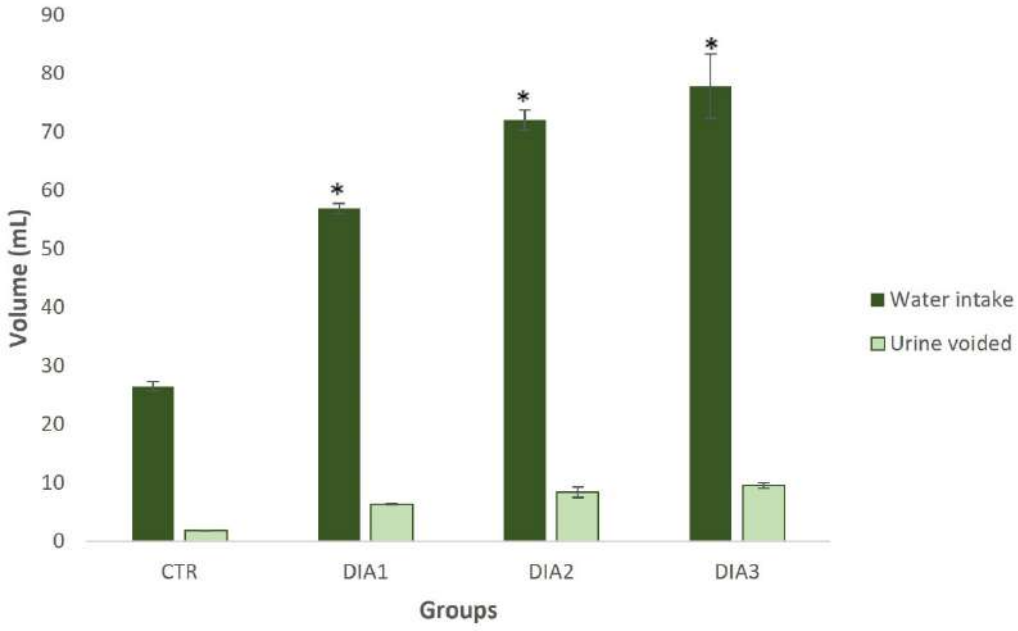

Figure 6. Fluid intake and output of control and test rats in mg/dL

Note: N=7, CTR=Control, DIA1=60 mg/kg STZ once, DIA2=60 mg/kg STZ two days consecutively, DIA3=60 mg/kg STZ three days consecutively. *P<0.005 from CTR.

Excess water loss through frequent urination decreases water content and increases the salt content in the body. This stimulates the hypothalamus's thirst center, increasing water intake (Deshmukh & Jain, 2015; Sembulingam, 2012). This present study showed a significant increase in the volume of water intake in the diabetic rats compared to the non-diabetic rats, which is in tandem with the findings of Alina et al. (2015).

Polyuria is excessive or frequent urination experienced by diabetic individuals, and it is the most common sign of diabetes (Mukthar et al., 2020). Previous studies by Akbarzadeh et al. (2007) showed that there was a significant increase in the volume of urine voided in streptozotocin-induced diabetic rats; in agreement with this claim, this present study also indicates that there is a significant increase in the volume of urine voided by the diabetic rats across the test group compared to non-diabetic (control) group.

Polyphagia arises due to the body's reaction to a lack of glucose, which has been lost because of polyuria, thus starving the body's cells (Mukthar et al., 2020). Previous studies showed increased food intake in streptozotocin-induced diabetic rats (Akbarzadeh et al., 2007; Alina et al., 2015; Wang-Fischer & Garyantes, 2018; Wang et al., 2022), aligning with the findings of this present study. However, there was no significant difference in the quantity of feed consumed by test group DIA1 compared to the control group. In contrast, the quantity of feed intake was significantly high in test group DIA3 throughout the study period, while the feed in- take in test group DIA2 was only significant in week two.

The consistency and appearance of the fecal materials, cleanliness of the coat, and the perineum are used to characterize intestinal dysfunction (diarrhea) in STZ-induced diabetic rats (Wang-Fischer & Garyantes, 2018). The result from this study showed that the diabetic test group and the control group were not diarrheic as their feces were well formed and not blood-stained; they also had a clean coat and non-matted perineum, suggesting normal gastrointestinal function as against the claim of Wang-Fischer & Garyantes (2018). Also, there was no significant difference in the quantity of feces defecated by the diabetic test group compared to the control group throughout this study.

This study discovered that test group DIA1 had a high recovery rate from diabetes at 71.4%, and (5 out of 7) rats in this group recovered within the three weeks of the research. In contrast, rats in test groups DIA2 and DIA3 did not recover during the study. This result agrees with the findings of Wang-Fischer & Garyantes (2018) that rats can recover from streptozotocin-induced diabetes.

Conclusion

This study shows that the dose regimen of 60 mg/kg STZ administered twice (24 hours apart) sustains diabetes for 21 days. We recommend using this dose regimen in STZ-induced diabetic studies in male Wistar rats.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Animal Ethics Committee of Federal University of Agriculture (Code: FUNAAB/COLVET/CREC/2022/02/03).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Methodology: Temtope Ajala and Oluwatodimu Adewole Adekoya; Statistical analysis: Oluwatodimu Adewole Adekoya and Adenike Iyabo Adeleye; Conceptualization and writing the original draft: Olushola Emmanuel Adeleye; Review and editing: Adenike Iyabo Adeleye and Olushola Emmanuel Adeleye.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors thank the Federal University of Agriculture, Abeokuta, management for allowing us to conduct this study.

References

Adeleye, O. E., Aboajah, N. A., Adeleye, A. I., Sogebi, E. A. O., Mshelbwala, F. M., & Adetomiwa, A. S., et al. (2019). Annona muricata Linn. ethanolic leaf extract ameliorates reproductive complications in streptozotocin-induced diabetic Wistar rats. *Journal of Natural Sciences Engineering and Technology, 18* (1), 166-175. [Link]

Adeleye, O. E., Aladeyelu, O. T., Adebiyi, A. A., Adeleye, I. A., Adetomiwa, A. S., & Apantaku, J. T., et al. (2020a). Ameliorative effects of Psidium guajava ethanolic leaf extract on streptozotocin-induced diabetic reproductive dysfunctions in male Wistar rats. *Alexandria Journal of Veterinary Science, 66* (1), 1-9. [DOI:10.5455/ajvs.101286]

Adeleye, O., Emmanuel, O. K. O. H., Adeleye, A., Mshelbwala, F. M., Adetomiwa, A., & Apantaku, J., et al. (2020). Ameliorative effects of Allium cepa Linn. scaly leaves extract on reproductive dysfunctions in streptozotocin-induced diabetic Wistar rats. *Journal of Istanbul Veterinary Sciences, 4*(3), 136-144. [DOI:10.30704/httpwww-jivs-net.811491]

Akbarzadeh, A., Norouzian, D., Mehrabi, M. R., Jamshidi, S.h, Farhangi, A., & Verdi, A. A., et al. (2007). Induction of diabetes by streptozocin in rats. *Indian Journal of Clinical Biochemistry, 22*(2), 60-64. [DOI:10.1007/BF02913315] [PMID]

Alina, S., Marcel, P., Alina, M., Ciprian, F., Adriana, V., & Doina, G., et al. (2015). Wistar rats with long-term streptozotocin-induced type 1 diabetes mellitus replicate the most relevant clinical, biochemical, and hematologic features of human diabetes. *Revista Română de Medicină de Laborator, 23*(3), 263-274. [DOI:10.1515/rrlm-2015-0028]

American Diabetes Association. (2014). Standards of medical care in diabetes - 2014. *Diabetes Care, 37*(Supplement 1), S14-S80. [PMID]

Baig, M. A., & Panchal, S. S. (2019). Streptozotocin-induced diabetes mellitus in neonatal rats: an insight into its applications to induce diabetic complications. *Current Diabetes Review, 16*(1), 26-39. [DOI:10.2174/1573399815666190411115829] [PMID]

Basu, R., Schwenk, W. F., & Rizza, R. A. (2004). Both fasting glucose production and disappearance are abnormal in people with "mild" and "severe" type 2 diabetes. *American Journal of Physiology. Endocrinology and Metabolism, 287*(1), E55–E62. [DOI:10.1152/ajpendo.00549.2003] [PMID]

Blonde, L., Umpierrez, G. E., Reddy, S. S., McGill, J. B., Berga, S. L., & Bush, M., et al. (2022). American Association of Clinical Endocrinology Clinical Practice Guideline: Developing a Diabetes Mellitus Comprehensive Care Plan-2022 Update. *Endocrine Practice, 28*(10), 923-1049. [DOI:10.1016/j.eprac.2022.08.002] [PMID]

Brown, E., Wilding, J. P. H., Barber, T. M., Alam, U., & Cuthbertson, D. J. (2019). Weight loss variability with SGLT2 inhibitors and GLP-1 receptor agonists in type 2 diabetes mellitus and obesity: Mechanistic possibilities. *Obesity Reviews, 20*(6), 816-828. [DOI:10.1111/obr.12841] [PMID]

Byrne, F. M., Cheetham, S., Vickers, S., & Chapman, V. (2015). Characterisation of pain responses in the high fat diet/streptozotocin model of diabetes and the analgesic effects of anti-diabetic treatments. *Journal of Diabetes Research, 2015,* 752481. [DOI:10.1155/2015/752481] [PMID]

Chao, P. C., Li, Y., Chang, C. H., Shieh, J. P., Cheng, J. T., & Cheng, K. C. (2018). Investigation of insulin resistance in the popularly used four rat models of type-2 diabetes. *Biomedicine &Pharmacotherapy, 101,* 155-161. [DOI:10.1016/j.biopha.2018.02.084] [PMID]

Cheraghi, J., Zargooshi, M., Kridhchi, P., &Nasri, S. (2021). Effects of parsley (petroselinum crispum) hydroalcoholic extract on spermatogenesis and pituitary- gonadal axis in streptozotocin-induced diabetic male rat. *Iranian Journal of Veterinary Medicine, 15*(4), 411-422. [Link]

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., & Ohlrogge, A. W., et al. (2018). IDF diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice, 138,* 271-281. [DOI:10.1016/j.diabres.2018.02.023] [PMID]

da Rocha Fernandes, J., Ogurtsova, K., Linnenkamp, U., Guariguata, L., Seuring, T., & Zhang, P., et al. (2016). IDF diabetes Atlas estimates of 2014 global health expenditures on diabetes. *Diabetes Research and Clinical Practice. 117,* 48-54. [DOI:10.1016/j.diabres.2016.04.016] [PMID]

Deeds, M. C., Anderson, J. M., Armstrong, A. S., Gastineau, D. A., Hiddinga, H. J., & Jahangir, A., et al. (2011). Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. *Laboratory Animals, 45*(3), 131-140. [DOI:10.1258/la.2010.010090] [PMID]

Deshmukh, C. D., & Jain A. (2015). Diabetes mellitus: A review. *International Journal of Pure and Applied Biosciences, 3*(3), 224-230. [Link]

Eitah, H. E., Maklad, Y. A., Abdelkader, N. F., Gamal El Din, A. A., Badawi, M. A., & Kenawy, S. A. (2019). Modulating impacts of quercetin/sitagliptin combination on streptozotocin-induced diabetes mellitus in rats. *Toxicology and Applied Pharmacology, 365,* 30-40. [DOI:10.1016/j.taap.2018.12.011] [PMID]

Forouhi, N. G., & Wareham, N. J. (2014). Epidemiology of diabetes. *Medicine (Abingdon, England: UK ed.), 42*(12), 698–702. [PMID]

Furman, B. L. (2021). Streptozotocin-Induced Diabetic Models in Mice and Rats. *Current Protocols, 1*(4), e78. [PMID]

Genuth, S., Alberti, K. G., Bennett, P., Buse, J., Defronzo, R., & Kahn, R., et al. (2003). Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care, 26*(11), 3160–3167. [DOI:10.2337/diacare.26.11.3160] [PMID]

Gundala, N. K. V., Naidu, V. G. M., & Das, U. N. (2018). Amelioration of streptozotocin-induced type 2 diabetes mellitus in Wistar rats by arachidonic acid. *Biochemistry and Biophysical Research Communications, 496*(1), 105–113. [DOI:10.1016/j.bbrc.2018.01.007] [PMID]

Holt, R., Cockram, C., Flyvbjerg, A., & Goldstein, B. (2010). *Textbook of Diabetes*. Blackwell Publishing. [Link]

Irannejad, A., Khatamsaaz, S., & Mokhtari, M. J. (2022). Effect of hydro-alcoholic extract of rosemary on lipid profile and liver enzymes in male wistar rats fed with high-fat diet. *Iranian Journal of Veterinary Medicine.* [Unpublished]. [Link]

Kasuga, M. (2006). Insulin resistance and pancreatic β cell failure. *The Journal of Clinical Investigation, 116*(7), 1756-1760. [DOI:10.1172/JCI29189] [PMID]

Kintoko, K., Qingwei, W., Xing, L., Ni Zheng, X., & Renbin, H. (2014). Diabetogenic activity of streptozotocin on Kunming strain mice as animal model of diabetes mellitus. *Journal of Pharmaceutical and Biological Science, 9*(1), 48-53. [DOI:10.9790/3008-09134853]

Kumar, P., & Clark, M. (2002). *Textbook of clinical medicine*. London: Edition, Saunders. [Link]

Lenzen, S. (2008). The mechanism of alloxan and streptozotocin diabetes. *Diabetologia, 51*(2), 216-226. [DOI:10.1007/s00125-007-0886-7] [PMID]

López-Soldado, I., & Herrera, E. (2003). Different diabetogenic' response to moderate doses of streptozotocin in pregnant rats, and its long-term consequences in the offspring. *Experimental Diabesity Research, 4*(2), 107–118. [DOI:10.1155/EDR.2003.107] [PMID]

Mahmoud, A. A., Zuhair, B. A., Alzaben, K. A., Abu-Halaweh, S. A., Mohamed, K. A., & Jaafar, A., et al. (2009). Induction of diabetes mellitus in rats using intraperitoneal streptozotocin: A comparison between 2 strains of rats. *European Journal of Scientific Research, 32*(3), 398-402. [Link]

Meigs, J. B., Nathan, D. M., D'Agostino, R. B., Sr, Wilson, P. W., & Framingham Offspring Study. (2002). Fasting and post challenge glycemia and cardiovascular disease risk: The Framingham offspring study. *Diabetes Care, 25*(10), 1845-1850. [DOI:10.2337/diacare.25.10.1845] [PMID]

Moghtadaei Khorasgani, E., & Khani, A. (2021). Investigating the effect of hydroalcoholic extract of eryngos on plasma concentration of blood glucose, blood cells and pancreatic tissue in diabetic rats. *Iranian Journal of Veterinary Medicine, 15*(4), 440-451. [DOI:10.22059/IJVM.2021.311523.1005134]

Mukhtar, Y., Galalain, A., and Yunusa, U. (2020). A modern overview on diabetes mellitus: A chronic endocrine disorder. *European Journal of Biology, 5*(2), 1-14. [DOI:10.47672/ejb.409]

Nagappa, A. N., Thakurdesai, P. A., Venkat Rao, N., & Singh, J. (2003). Antidiabetic activity of Terminalia catappa Linn fruits. *Journal of Ethnopharmacology, 88*(1), 45–50. [DOI:10.1016/S0378-8741(03)00208-3] [PMID]

Nelson, R. W., & Reusch, C. E. (2014). Animal models of diseases: Classification and etiology of diabetes in dogs and cats. *Journal of Endocrinology, 222*(3), T1-T9. [DOI:10.1530/JOE-14-0202] [PMID]

Olokoba A. B., Obateru, O. A, Olokoba, L. B. (2012). Type 2 diabetes mellitus: A review of current treatment trend. *Oman Medical Journal, 27*(4), 269-273. [DOI:10.5001/omj.2012.68] [PMID]

Rand, J. S. (2020). Diabetes mellitus in dogs and cats. In: D. S. Bruyette, N. Bexfield, J. D. Chretin, L. Kidd, S. Kube, & C. Langston (Eds.), *Clinical small animal internal medicine* (pp. 93-102). New Jersey: John Wiley & Sons, Inc. [Link]

Robert, A. R. (2010). Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: Implications for therapy. *Diabetes, 59*(11), 2697-2707. [DOI:10.2337/db10-1032] [PMID]

Rosqvist, F., Kullberg, J., Ståhlman, M., Cedernaes, J., Heurling, K., & Johansson, H. E., et al. (2019) Overeating saturated fat promotes fatty liver and ceramides compared with polyunsaturated fat: A randomized trial. *Journal of Clinical Endocrinology and Metabolism, 104*(12), 6207-6219. [DOI:10.1210/jc.2019-00160] [PMID]

Rudge, M. V., Piculo, F., Marini, G., Damasceno, D. C., Calderon, I. M., & Barbosa, A. P. (2013). [Translational research in gestational diabetes mellitus and mild gestational hyperglycemia: current knowledge and our experience 9 Portuguese)]. *Arquivos Brasileiros de Endocrinologia e Metabologia, 57*(7), 497-508. [DOI:10.1590/S0004-27302013000700001] [PMID]

Scheen, A. J. (2003). Pathophysiology of type 2 diabetes. *Acta Clinica Belgica, 58*(6), 335-341. [DOI:10.1179/acb.2003.58.6.001] [PMID]

Schwab, U., Lauritzen, L., Tholstrup, T., Haldorssoni, T., Riserus, U., & Uusitupa, M., et al. (2014). Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: A systematic review. *Food & Nutrition Research, 58*, 10.3402/fnr.v58.25145. [DOI:10.3402/fnr.v58.25145] [PMID]

Sembulingam, K., & Sembulingam, P. (2012). *Essentials of medical physiology*. New Delhi: Jaypee Brothers Medical Publishers Pvt. Limited. [Link]

Shahsavari, M., Norouzi, P., Kalalianmoghaddam, H., & Teimouri, M. (2023). Effects of Kudzu Root on Oxidative Stress and Inflammation in Streptozotocin-Induced Diabetic Rats. *Iranian Journal of Veterinary Medicine, 17*(4), 401-408. [Link]

Svane, M. S., Jørgensen, N. B., Bojsen-Møller, K. N., Dirksen, C., Nielsen, S., & Kristiansen, V. B., et al. (2016). Peptide YY and glucagon-like peptide-1 contribute to decreased food intake after Roux-en-Y gastric bypass surgery. *International Journal of Obesity (Lond), 40*(11), 1699-1706. [DOI:10.1038/ijo.2016.121] [PMID]

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiology Research, 50*(6), 536-546. [Link]

Thomas, C. C., & Philipson, L. H. (2015). Update on diabetes classification. *The Medical Clinics of North America, 99*(1), 1–16. [DOI:10.1016/j.mcna.2014.08.015] [PMID]

Wang-Fischer, Y., & Garyantes, T. (2018). Improving the reliability and utility of streptozotocin-induced rat diabetic model. *Journal of Diabetes Research, 2018,* 8054073. [DOI:10.1155/2018/8054073] [PMID]

Wang, J., Jiang, J., Zhao, C., Shan, H., Shao, Z., & Wang, C., et al. (2022). The protective effect of theaflavins on the kidney of mice with type II diabetes mellitus. *Nutrients, 15*(1), 201. [PMID]

Wei, M., Ong, L., Smith, M. T., Ross, F. B., Schmid, K., & Hoey, A. J., et al. (2003). The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. *Heart, Lung and Circulation, 12*(1), 44-50. [DOI:10.1046/j.1444-2892.2003.00160.x] [PMID]

This Page Intentionally Left Blank