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Effective Dose Regimen of STZ for STZ-induced Diabetes in a Rat Model

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Abstract

BACKGROUND: Diabetes mellitus (DM) is a metabolic illness, defined by elevated level of blood sugar because of a problem with insulin synthesis, action, or both. Various clinical signs follow DM, majorly hyperglycemia, polydipsia, polyuria, and polyphagia. Worldwide prevalence is high and predicted to rise to 592 million by 2035. Animal models are used in the study of diabetes due to ethical issues. Streptozotocin (STZ) model is frequently used but has poor dependability 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: 28 male Wistar rats (160 - 190 g) were randomly divided into 4 groups (n=7) and monitored for 21 days after diabetes induction with STZ: Control (CTR), diabetics: DIA1 (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 $P \leq 0.05$ for significance.

RESULTS: There was significant ($p<0.05$) decrease in body weight of the diabetics (-15.53±1.2, -26.8±1.2, -28.5±1.9%) compared to the CTR (10.5±2.5%). There was 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 diabetics 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, 9.58±0.50 mL) and voided feces (10.4±0.26, 11.7±0.43, 8.5±0.17 g) in the diabetics 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).

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

KEYWORDS: dose regimen, diabetes, streptozotocin, Wistar rats

1. Introduction

A metabolic illness known as diabetes mellitus (DM) is characterized by an increased level of sugar in the blood (hyperglycemia) resulting from problems with insulin synthesis, action, or both (ADA, 2014; Thomas *et al.,* 2015). The following criteria are used to diagnose DM: fasting plasma glucose concentration ≥126 mg/dL (after ≥8 h of an overnight fast), or plasma glucose (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, either from the same sample or from two different samples drawn on successive days. But a glucose reading of greater than 200 mg/dL in the presence of DM symptoms confirms the condition (Blonde *et al.*, 2022).

Chronic hyperglycemia, polydipsia, polyuria, polyphagia, impaired vision, unexplained weight loss, lack of energy, diabetic ketoacidosis, hyperosmolar and hyperglycemic non-ketotic

65 syndrome, glycosuria, and weariness are all major clinical consequences of diabetes mellitus (Kumar *et al.,* 2002; ADA, 2014; Rand, 2020).

With variations in frequency among various ethnic groups, there were more than 425 million cases of DM worldwide in 2017, and by 2045, it is predicted that there would be 629 million cases with type 2 diabetes accounting for most (>85%) of the total DM prevalence (Cho *et al*.,
70 2018; Forouhi and 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 to foot ulcers, limb decapitation, and Charcot joints, and autonomic neural disorder, which can lead to gastrointestinal, genitourinary, cardiovascular, and sexual dysfunctions. Artery hardening,
75 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 health care systems around the globe (da Rocha Fernandes *et al*., 2016).

The two primary classifications for diabetes are type 1 diabetes and type 2 diabetes. Both emerge
80 from complex gene-environment interactions, but they have different pathophysiology. 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 percent to 1.2 percent. 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 and Reusch, 2014; Rand, 2020). Streptozotocin (STZ; N-nitro derivative of glucosamine) occurs naturally, and it is a broad-spectrum antibiotic which 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—more than 17,000 STZ listings on PubMed—reviewers who are unfamiliar with a model of STZ-induced diabetes may find it challenging to accurately plan new investigations. 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 (i.p.) or intravenous (i.v.) injection of streptozotocin, experimental diabetic mellitus can be brought on in 2 to 4 days (Wei *et al*., 2003, Furman, 2021). 45 mg/kg, intraperitoneal STZ injection induced 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) or followed by single injection of STZ 45 mg/kg, induced diabetes in rats (Byrne *et al*., 2015). 60 mg/kg STZ injected once intraperitoneally will induce extensive necrosis of Langerhans islets beta cells, also a single 65 mg/kg intravenous (i.v.) injection or by two 50 mg/kg i.v. injections at 3 days apart; a single dose at 60 mg/kg i.p. (Moghtadaei et al., 2021); or a single dose at 65 mg/kg i.p. at 55 mg kg (Shahsavani *et al*., 2022); or a single dose at 65 mg/kg i.v.; or 50 mg/kg i.v. (Szkudelski, 2001; Deeds *et al*., 2011; Adeleye *et al*., 2019, 2020a, 2020b, Cheraghi *et al*., 2021). Different strains of research animal models react in differing ways to this injection, and this should be understandably noted (Mahmoud *et al*., 2009).

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

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 and Herrera, 2003), animal models must be used, to better comprehend the pathophysiology of diabetes (Rudge, 2013; Baig and 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. To better understand the right dose regimen, pathophysiological mechanisms, and clinical symptoms in streptozotocin-induced diabetic rats, this research was conducted.

2. 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 COLVET, FUNAAB, Abeokuta, Ogun State, Nigeria after being obtained from the Teaching and Research Animal House, University of Ibadan. They were kept in 12-hour light/12-hour darkness conditions, fed a conventional rat diet, and always had access to water, unless otherwise stated. When conducting this study, we adhered to the rules set forth by the Federal University of Agriculture, Abeokuta's committee on animal care ethics and usage (FUNAAB/COLVET/CREC/2022/02/03).

Experimental procedure

The 28 rats were divided into four groups of seven by random selection, and groups DIA1 through DIA3 were given a once daily intraperitoneal doses of 60 mg/kg STZ [once (DIA1), twice (DIA2), and three times (DIA3)] to induce the diabetic state while group A was not treated. The animals were monitored daily (Figure 1 and 2).

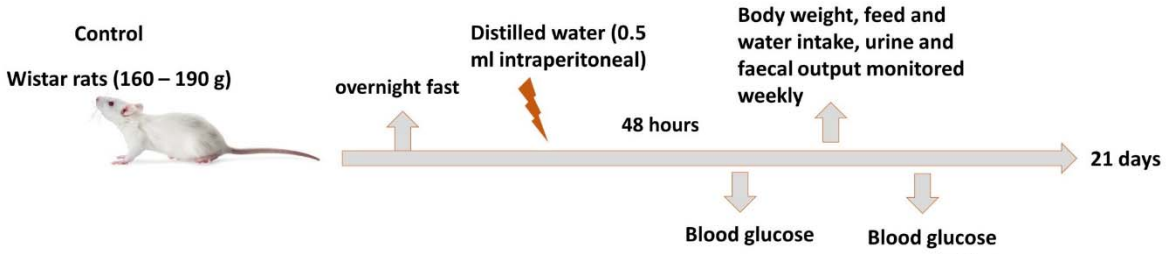

Figure 1: Control rats' protocols. N = 7, CTR = Control

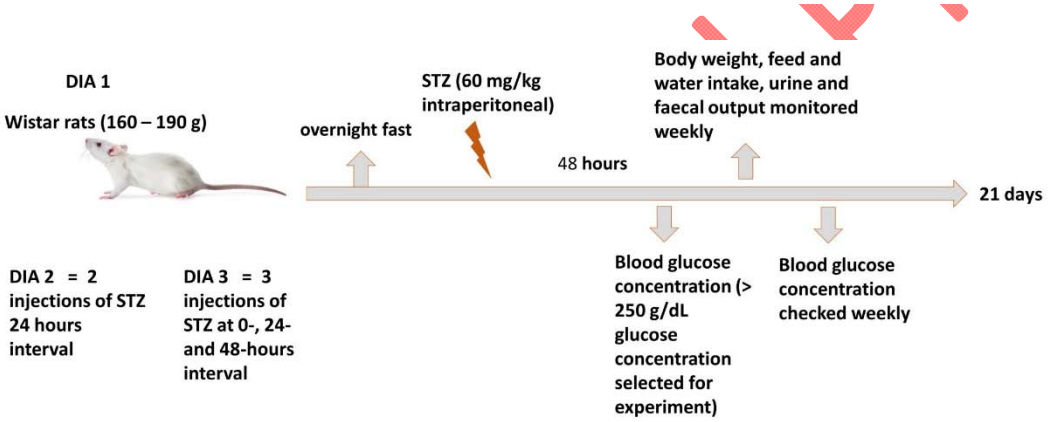

Figure 2: Test rats' protocols. N = 7, DIA1 = 60 mg/kg STZ once, DT2 = 60 mg/kg STZ two days consecutively, DT3 = 60 mg/kg STZ three days consecutively

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 method in mg/dL (Nagappa *et al*., 2003). Briefly, a drop of

blood obtained from the tail vein of the rats was placed on the test strip that was already 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. The test strips are loaded with enzymes which react with the glucose in the blood. There is a color change due to the reaction which the meter measures and translates into the concentration of glucose in mg/dL. Blood glucose values for each rat were repeated thrice and the average reading was used, and this 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, finally multiplied by 100.

% 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 measure the feed intake and fecal output (Camry®).

Data analysis

Data were collected tabulated and 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; Inpixon Inc., USA) was used for all analysis and *P* values less than 0.05 were significant.

3. 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 significant increase ($P<0.05$) in the fasting blood glucose of test group (DIA1, DIA2 and DIA3) compared to control group.

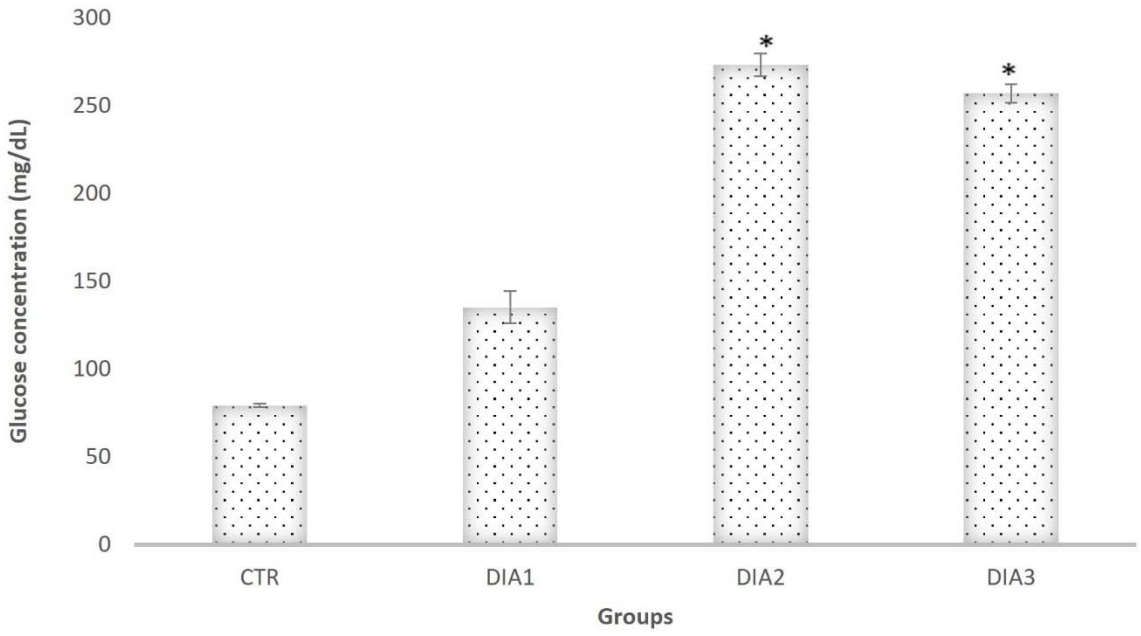

Figure 3: Fasting blood glucose level of control and test rats in mg/dL, N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DT2 = 60 mg/kg STZ consecutively, DT3 = 60 mg/kg STZ three days consecutively

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).

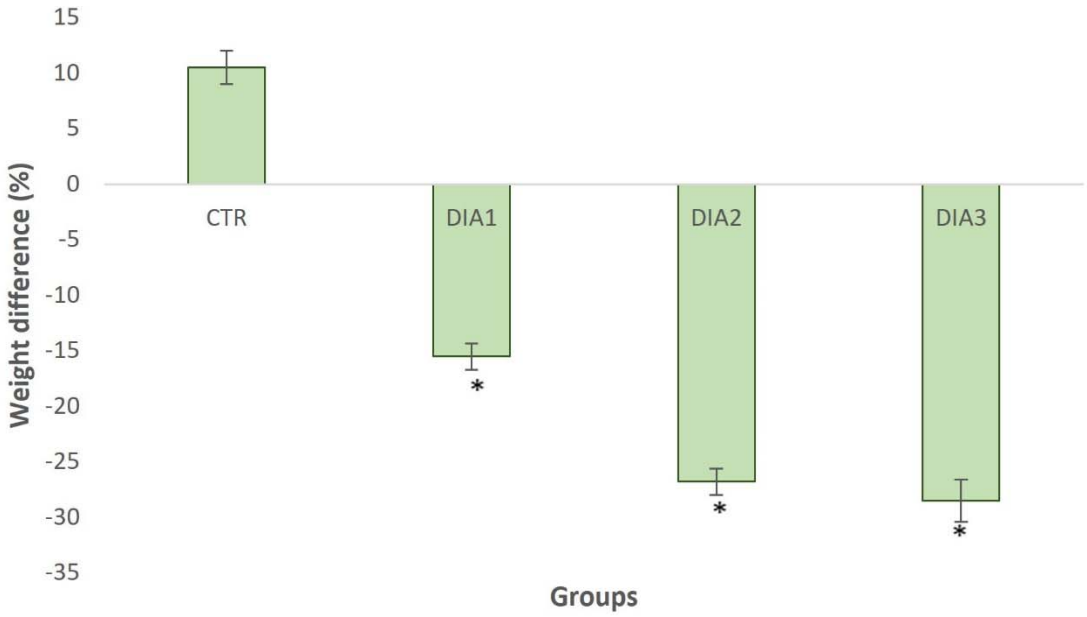

Figure 4: Weight difference of control and test rats in %, N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DT2 = 60 mg/kg STZ consecutively, DT3 = 60 mg/kg STZ three days consecutively

Feed intake

The intake of feed 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.

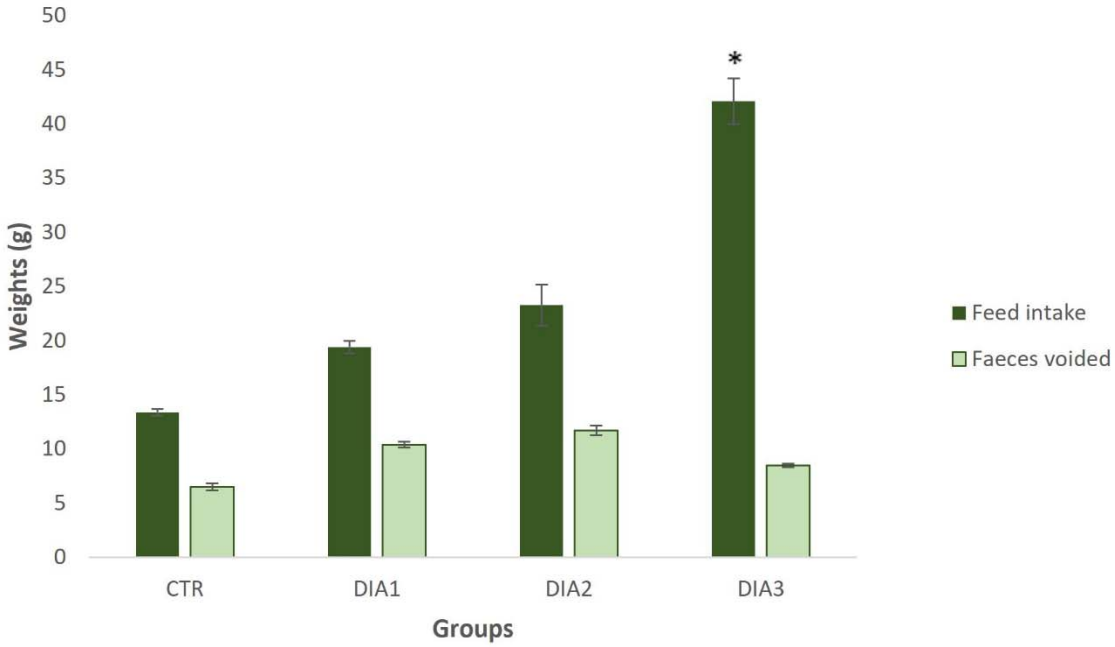

Figure 5: Feed intake and voided faeces of control and test rats in g, N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DT2 = 60 mg/kg STZ consecutively, DT3 = 60 mg/kg STZ three days consecutively

Intake and output of fluid

The intake and measured output of fluid by the control rats remained constant throughout the course of the experiment with intake always 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).

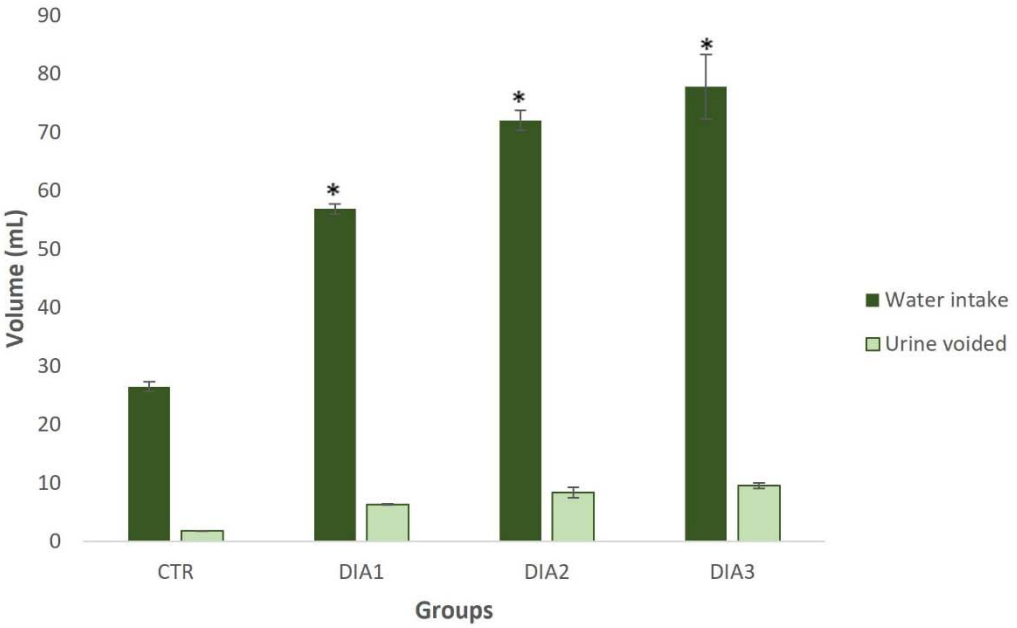

Figure 6: Fluid intake and output of control and test rats in mL, N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DT2 = 60 mg/kg STZ consecutively, DT3 = 60 mg/kg STZ three days consecutively

4. Discussion

The result of this research showed that rats that received two successive administrations of
195 streptozotocin 60 mg/kg intraperitoneally (DIA2) had a higher blood glucose level compared to the rats that received single dose (DIA1) and three successive doses of streptozotocin intraperitoneally (DIA3). This result contradicts the findings of Alina *et al.,* 2015 where they did not record any significant difference between the blood glucose level of rats that were 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.,* 2023), 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 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 urine output of the test rats was significantly increased which equates to loss of necessary electrolytes from the body and the increased water intake tends to reduce 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 feed 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 energy 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), but 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.

Excess loss of water through frequent urination decreases water content and increases the salt content in the body. This stimulates the thirst center in the hypothalamus which then increases the intake of water (Deshmukh and Jain 2015; Sembulingam, 2012). The result of this present study showed that there was 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 commonest sign of diabetes (Mukthar *et al.,* 2020). Previous studies by Akbarzadeh *et al.,* 2007 showed that there was significant increase in the volume of urine voided in streptozotocin induced diabetic rats, in agreement to this claim this present study also shows that there is 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 lack of glucose which has been lost because of polyuria, thus starving the body cells (Mukthar *et al.,* 2020). Previous studies showed that there was increased feed intake in streptozotocin induced diabetic rats (Akbarzadeh *et al.,* 2007; Alina *et al.,* 2015; Wang-Fischer and Garyantes, 2018; Wang *et al*., 2023), aligning with the findings

of this present study. However, there was no significant difference in the quantity of feed consumed by test group one (DIA 1) compared to the control group, while the quantity of feed intake was significantly high in test group three (DIA 3) throughout the study period while the feed intake test group two (DIA 2) was only significant in week two of the study.

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

In this study, it was discovered that test group 1 (DIA 1) had a high rate of recovery from diabetes at 71.4%, (5 out 7) rats in this group recovered within the three weeks period of the research while rats in test group DIA 2 and DIA 3 did not recover during the research and this result agrees with the findings of Wang-Fischer and Garyantes (2018) that rat is able recover in streptozotocin induced diabetes.

5. Conclusion

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

Acknowledgments

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