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Comparison of the effects of streptozotocin and alloxan at different doses and administration routes in a rat model of diabetes

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Abstract

Doses and route of administration in chemical diabetes modeling critically shape the performance of diabetes models, but head-to-head, dose-matched comparisons without additional drugs are limited. This study aimed to compare glycemic, body weight, and oxidative stress induced by two doses and two routes of administration of streptozotocin (STZ) and Alloxan (ALX) in male Wistar rats. A total of 56 male Wistar albino rats (200 – 250 g) were included in the study and randomized into eight groups (n = 7 per group). STZ and ALX at doses of 45 or 60 mg/kg were administered intravenously (i.v.) and intraperitoneally (i.p.). Blood glucose was measured at baseline and every 3 days for 21 days; body weight was monitored; and oxidative balance was assessed by Total oxidant status (TOS) and Total antioxidant status (TAS). Group and time effects were analyzed using repeated measures statistics using SPSS 30. All groups exceeded 200 mg/dL on day 3, confirming diabetes. Among the STZ arms, 60 mg/kg i.p. produced the highest glycaemia from day 9 to day 15, after which the 45 mg/kg i.v. arm reached comparable or higher values; the 45 mg/kg i.p. arm remained substantially lower throughout. In the ALX arms, dose was the primary determinant: 60 mg/kg i.p. produced the highest and most sustained hyperglycemia from day 6; whereas at 45 mg/kg blood glucose did not differ significantly between the i.v. and i.p. routes. Weight loss was greatest in the 60 mg/kg i.v. group. TOS was greater with ALX compared to STZ. TOS was lowest with 45 mg/kg i.v. ALX. TAS was higher with ALX than with STZ at 45 mg/kg, but not at 60 mg/kg. No mortality occurred over the 21-day period. In this dose-matched comparison, dose is the primary determinant of model strength, and route of administration modulates the response more for ALX than for STZ. Diabetes was diagnosed starting on day 3 with both doses and routes of administration. In practice, 60 mg/kg i.p. ALX and STZ produce consistently high blood glucose levels suitable for diabetes induction, while i.v. administration promotes early peaks and i.p. administration promotes a sustained glycemic load. These findings provide practical guidance for selecting agent, dose, and route in future preclinical diabetes studies.

Keywords: Streptozotocin, alloxan, rat, diabetes model, dose-response, oxidative stress

Introduction

Elucidation of the pancreas–insulin relationship has led hyperglycemia research to adopt non-surgical pancreatic ablation and the use of beta-cell–specific toxins. In experimental diabetes models induced by chemical agents, diabetogenic toxins such as alloxan (ALX), streptozotocin (STZ), zinc chelators (8-hydroxyquinoline, dithizone), the rodenticide Vacor, and dietary nitrosamines have been tested [1]. Today, the most commonly used are STZ and ALX. It has been reported that STZ causes less damage to structures other than beta cells than ALX; that higher blood glucose values are achieved with ALX; and that ketosis is more frequent [2].

ALX is a classic diabetogenic agent widely used to generate an insulin-dependent (type-1–like) diabetes phenotype in experimental animals. ALX selectively damages pancreatic islet β-cells. The diabetogenic effect requires parenteral delivery—intravenous (i.v.), intraperitoneal (i.p.) or subcutaneous (s.c.)—and the dose needed for reliable induction varies with species and with the route employed [3]. Its effect in the pancreas is through the rapid uptake of endogenous reducing agents by β cells, which direct the reduction reactions. The superoxide formed as a result of the reaction of the reducing agents releases iron from ferritin, moving it between Fe^{2+}/Fe^{3+} and forming H_2O_2 via dismutation. In the presence of Fe^{2+} and H_2O_2, reactive oxygen species (ROS)

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are formed. ROS also target islet cell DNA, and ALX causes damage and degradation in β cell DNA [4].

STZ is a naturally occurring chemical; it is used in animal models to induce type 1 diabetes and, with multiple low-dose protocols, for type 2 diabetes as well. STZ is a monofunctional nitrosourea derivative [5]. STZ inhibits DNA synthesis in mammalian and bacterial cells; in bacteria, it reacts to cause degeneration and degradation of DNA. STZ enters pancreatic cells via glucose transporter 2 (GLUT2) and causes DNA alkylation. It also stimulates poly (ADP-ribose) polymerase (PARP) activation and nitric oxide release; as a result, pancreatic β-cells are destroyed by necrosis and insulin-dependent diabetes is induced [6].

Across the literature, investigators have used a range of doses to establish diabetes models. For ALX and STZ, dose windows reported in different species and the most common regimens are often summarised narratively. In rats, ALX and STZ are the agents most frequently employed to induce experimental diabetes. ALX is typically administered at 30 – 65 mg/kg i.v., 75 – 250 mg/kg i.p., and 100 – 200 mg/kg s.c., whereas STZ is given at 40 – 80 mg/kg i.v., 35 – 150 mg/kg i.p., and 60 – 100 mg/kg s.c. For type I diabetes models, widely used schemes include a single i.v. dose of 40 – 65 mg/kg or a single i.p. dose of ≥ 150 mg/kg for ALX; for STZ, single doses of 35 – 80 mg/kg delivered i.v./i.p./s.c. (or 40 mg/kg intramuscularly) are common. In type II paradigms, ALX is used as a single 120 mg/kg i.p. dose in adult rats and 200 – 250 mg/kg i.p. in neonates; STZ-based approaches employ a single 100 mg/kg dose in neonates, while in adults low-dose protocols combined with nicotinamide or a high-fat diet are preferred [7]. In our study, the chosen ALX doses fall within the effective 40 – 65 mg/kg i.v. range in rats, yielding reproducible hyperglycemia without increasing mortality [8]. In rats, STZ between 35 – 80 mg/kg produces β-cell selective, stable type-1-like hyperglycemia [9]. A 45 mg/kg ALX dose reliably establishes permanent diabetes in rats while leaving hepatic microsomal protein, cytochrome P450 and cytochrome b5 levels unchanged [10]. In rats receiving 45 mg/kg STZ, a diabetes phenotype has been obtained with an additional injection [11]. A single 60 mg/kg STZ dose has also been used to induce oxidative stress and diabetes [12]. A nominally sub-diabetogenic ALX dose of 60 mg/kg has nonetheless produced persistent hyperglycemia in animals [13]. Taken together, these reports support the use of STZ and ALX in the 45 – 60 mg/kg range for diabetes induction in rats, whether as single i.v./i.p. injections [10,12,13] or as part of multi-dose or combination protocols, including high-fat diet co-treatment and nicotinamide co-administration [11,14,15]. The aim of this study was to comparatively evaluate the dose-dependent effects of ALX and STZ in rat models of experimental diabetes by assessing glycemic status together with oxidative stress parameters. Because both agents induce pancreatic β-cell injury through mechanisms involving oxidative damage, total oxidant status (TOS) and total antioxidant status (TAS) were measured to compare the systemic redox alterations associated with each model beyond hyperglycemia alone.

Material and Methods

Experimental Method

Fifty-six male Wistar albino rats were studied (7 per group; body weight 200 – 250 g). Sample size was determined a priori with G*Power 3.1.9.4: assuming an effect size f = 0.55, α = 0.05, and target power of 0.80, a single–time-point one-way analysis of variance (ANOVA) with eight groups yields an achieved power of ~0.81 at N = 56. Animals were housed in the Experimental Animal Laboratory of Ankara Medipol University under standard conditions (12:12 h light–dark cycle, 45 – 65% relative humidity, 21 ± 2°C) with chow and water available ad libitum. All procedures were reviewed and approved by the Ankara Medipol University Animal Experiments Local Ethics Committee (HADYEK) (03 December 2025; Protocol No. AMUHADYEK-24; Document No. E-21265738-20.01-11797).

Rats were randomly assigned to eight arms defined by diabetogenic agent (STZ or ALX), dose (45 or 60 mg/kg), and route (i.v. or i.p.): STZ 60 mg/kg i.v.; STZ 60 mg/kg i.p.; STZ 45 mg/kg i.v.; STZ 45 mg/kg i.p.; ALX 60 mg/kg i.v.; ALX 60 mg/kg i.p.; ALX 45 mg/kg i.v.; and ALX 45 mg/kg i.p. IV injections were performed via the tail vein. IP injections were carried out in the lower right abdominal quadrant. For all procedures, animals were fasted for 24 h with free access to water. STZ was prepared to body-weight–adjusted doses in 50 mM citrate buffer (pH 4.5) and kept on ice until use. ALX was freshly dissolved in cold sterile distilled water and maintained below 4°C. Throughout the study, standard chow and water remained unrestricted.

Diabetes was defined as > 200 mg/dL from day 3 onward after dosing by measuring tail-capillary glucose with a handheld glucometer (scalpel tail nick); animals with blood glucose > 200 mg/dL were classified as diabetic [16]. Following diabetes confirmation, the experiment continued for 21 days. During this period, blood glucose and body weight were monitored every three days.

Analysis of Total Oxidant-Antioxidant Parameters

Twenty-one days after diabetes induction, rats were euthanised. Animals were anesthetized using ketamine (60-80 mg/kg, i.p.) combined with xylazine (5 – 10 mg/kg, i.p.). Aortic blood was collected for biochemical analysis. Whole blood was centrifuged at 1,000 × g for 15 minutes at 4°C, after which plasma was separated, aliquoted, and stored at -80°C until testing. Systemic oxidative status was assessed by determining plasma TOS and TAS indices. TOS was quantified with a commercial kit (Rel Assay, Türkiye). In this assay, oxidants in the sample convert the Fe^{2+}–o-dianisidine complex to Fe^{3+}; in an acidic medium the ferric ions form a coloured complex with xylenol orange. Absorbance at 530 nm on a Mindray BS-400 analyser is proportional to the TOS, and values are reported as μmol H_2O_2 equivalents per litre [17].

Plasma TAS was quantified with a commercial kit (Rel Assay, Türkiye). The assay hinges on the reduction of the chromogenic 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS•+) by antioxidants present in the sample, which decreases colour intensity. Absorbance was recorded at 660 nm

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on a Mindray BS-400 analyser, and values were calculated against a Trolox standard and expressed as mmol Trolox equivalents per litre [18].

The Oxidative Stress Index (OSI), reflecting the systemic oxidant–antioxidant balance, was calculated as the ratio of TOS to TAS using the formula OSI = (TOS / TAS) × 100, in line with Erel's methodology [17,18]. Higher OSI values indicate a greater predominance of oxidative species relative to antioxidant capacity.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Effects of agent (STZ vs ALX), dose (45 vs 60 mg/kg), and route (i.v. vs i.p.) were tested using a repeated-measures ANOVA. OSI was analysed using two-way ANOVA (dose × route) with Tukey post-hoc tests, separately for STZ and ALX. Pre-specified STZ–ALX comparisons within the same dose–route combination were evaluated with independent-samples t tests. Longitudinal outcomes were analysed by repeated-measures ANOVA, with Tukey-adjusted post-hoc tests when the omnibus result was significant. Assumptions were checked using Shapiro–Wilk (normality) and Levene's test (homogeneity). All tests were two-sided with $\alpha = 0.05$. Analyses were performed in SPSS v30, with additional computations in Python 3.11 (SciPy, statsmodels).

Results

Blood glucose levels, body weight, and oxidative-stress parameters were evaluated in groups receiving STZ or ALX at different doses and via different routes. Throughout the experiment, blood glucose and body weight were recorded eight times at three-day intervals. From day 3 onward, all groups showed a significant rise in blood glucose ($p < 0.001$). The 60 mg/kg STZ i.p. group showed the highest glycaemia among the STZ arms from day 9 to day 15; by days 18 and 21 the 45 mg/kg i.v. arm reached comparable or higher values. The 45 mg/kg i.p. group remained markedly lower than the other STZ arms throughout, plateauing between approximately 345 and 442 mg/dL (Table 1, Figure 1).

The 60 mg/kg ALX i.p. cohort exhibited blood glucose levels that exceeded all other groups ($p < 0.01$). No difference was detected between the 45 mg/kg ALX i.v. and i.p. regimens ($p > 0.05$). At 60 mg/kg, ALX given i.v. produced higher values than either 45 mg/kg group ($p < 0.05$) (Table 2; Figure 2).

Table 1. Blood glucose levels (mg/dL; mean ± SD) in Streptozotocin groups at different doses and administration routes

Day	60 mg/kg iv	60 mg/kg ip	45 mg/kg iv	45 mg/kg ip
	Blood glucose level (mg/dL, mean ± SD)			
0	90.3 ± 7.3	92.7 ± 4.7	90.4 ± 5.0	89.0 ± 11.4
3	447.1 ± 73.8	459.4 ± 104.6	395.7 ± 44.6	389.9 ± 49.2
6	539.1 ± 51.4	521.7 ± 104.6	456.0 ± 74.9	344.9 ± 68.3
9	552.6 ± 43.5	595.1 ± 73.3	528.7 ± 61.2	369.1 ± 63.4
12	576.6 ± 35.4	655.3 ± 85.0	604.6 ± 105.8	398.7 ± 72.7
15	531.9 ± 34.7	641.6 ± 59.3	629.1 ± 88.7	378.7 ± 78.6
18	583.3 ± 43.1	586.7 ± 94.8	685.3 ± 83.9	400.1 ± 78.0
21	605.7 ± 76.7	680.3 ± 95.2	705.0 ± 86.9	442.0 ± 108.5

Blood glucose levels increased markedly from Day 3 onward in all streptozotocin-treated groups, with sustained hyperglycemia throughout the study period and the highest final values observed in the 60 mg/kg ip and 45 mg/kg iv groups

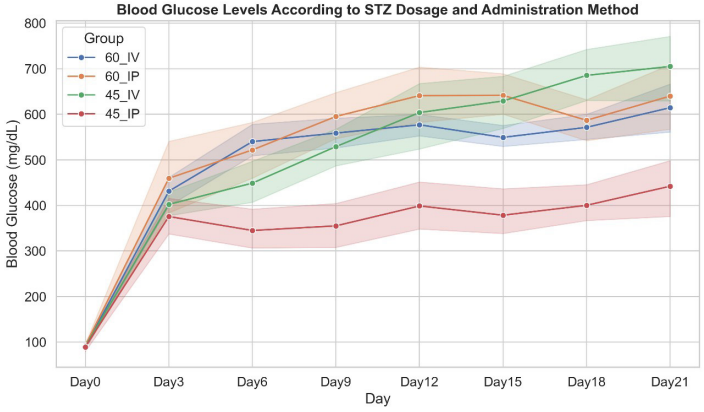

Figure 1. Blood glucose levels according to STZ dosage and administration method; Statistical difference is indicated as $p < 0.05$

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Table 2. Blood glucose levels (mg/dL; mean ± SD) in ALX groups at different doses and administration routes

Day	60 mg/kg iv	60 mg/kg ip	45 mg/kg iv	45 mg/kg ip
	Blood glucose level (mg/dL, mean ± SD)			
0	90.6 ± 3.7	90.4 ± 5.3	86.6 ± 7.3	86.0 ± 6.0
3	499.0 ± 90.4	477.4 ± 22.9	359.0 ± 66.8	340.1 ± 34.5
6	540.9 ± 70.5	571.7 ± 41.6	412.3 ± 53.6	412.8 ± 23.5
9	552.6 ± 89.8	586.3 ± 59.6	437.7 ± 57.7	447.0 ± 30.8
12	593.4 ± 83.7	677.1 ± 58.1	470.8 ± 81.4	498.9 ± 45.0
15	619.7 ± 73.4	711.9 ± 63.5	477.3 ± 80.1	531.6 ± 43.1
18	625.7 ± 82.0	730.5 ± 56.4	544.9 ± 91.6	574.1 ± 52.3
21	658.3 ± 92.4	736.6 ± 49.4	553.1 ± 103.8	584.4 ± 55.7

Blood glucose levels rose sharply from Day 3 in all alloxan-treated groups and remained elevated through Day 21, with the most pronounced and sustained hyperglycemia observed in the 60 mg/kg ip group

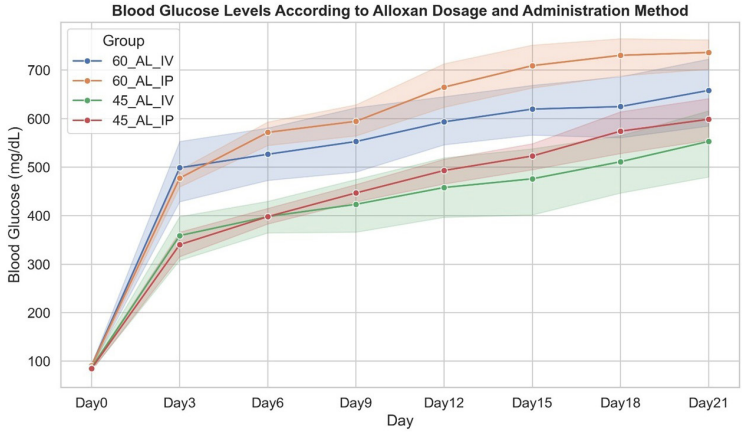

Figure 2. Blood glucose levels according to ALX dosage and administration method; Statistical difference is indicated as $p < 0.05$

Significant differences in blood glucose between the STZ and ALX groups were evident by day 3 ($p < 0.001$). By day 6, the 60 mg/kg ALX i.p. group exhibited markedly higher blood glucose than the ALX i.v., STZ i.v., and STZ i.p. groups ($p < 0.001$). The STZ i.p. group yielded slightly higher values than STZ i.v., with significance confined to days 9 and 12 ($p < 0.05$). Although ALX i.v. produced significantly higher blood glucose than the STZ groups early on ($p < 0.01$), after day 15 its levels remained below those of ALX i.p. ($p < 0.05$). Overall, ALX—via both i.v. and i.p. routes—induced a more pronounced and sustained hyperglycaemic effect than STZ, with the strongest effect in the 60 mg/kg ALX i.p. group ($p < 0.001$) (Figure 3).

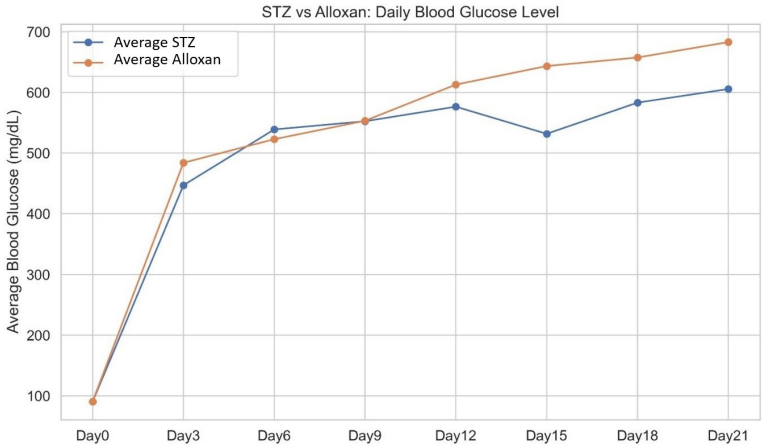

Figure 3. Comparison of average blood glucose levels between STZ and ALX groups over time; Statistical difference is indicated as $p < 0.05$

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Dose and route exerted a clear influence on body-weight loss. The most pronounced reduction ($p < 0.001$) was observed in the 60 mg/kg i.v. arms for both ALX and STZ. Moderate decreases ($p = 0.01$) occurred in the low-dose i.v. and high-dose i.p. groups, whereas the ALX 45 mg/kg i.p. group showed weak or borderline-significant loss ($p = 0.05$). Overall, the pattern followed a dose–route gradient, with i.v. > i.p. and 60 mg/kg > 45 mg/kg. These data indicate that high-dose i.v. administration -particularly ALX 60 mg/kg i.v.-produced the fastest and largest weight loss, while low-dose i.p. dosing yielded a more gradual, stable decline.

Across the 21-day follow-up, body weight decreased significantly in all experimental groups ($p < 0.001$). Moreover, the type of agent, dose, and administration route each had a marked effect on the magnitude of weight loss ($p < 0.001$), and a significant Time × Group interaction demonstrated that the rate of change differed among groups; declines were faster and more pronounced with i.v. than i.p. ($p < 0.001$) (Table 3 and Table 4).

TAS levels in the 60 mg/kg groups (i.v. and i.p.) were low and nearly identical, with no significant differences between them. By contrast, at 45 mg/kg, TAS levels increased markedly, and this rise was more pronounced with i.p. administration. A two-way ANOVA (dose × route) confirmed that the TAS elevation was statistically significant at the 45 mg/kg dose and with the i.p. route ($p < 0.05$) (Table 5).

Table 3. Mean body weight change in streptozotocin and ALX groups (0 – 21 Days)

Group	Δ Mean weight (g)	% Change (from day 0)	p value
STZ 60 IV	- 61.5 ± 18.4 g	- 27.6 %	**p < 0.001 ***
ALX 60 IV	- 72.6 ± 18.9 g	- 33.1 %	**p < 0.001 ***
STZ 45 IV	- 48.1 ± 13.3 g	- 21.8 %	**p ≈ 0.010 **
ALX 45 IV	- 53.5 ± 17.0 g	- 23.6 %	**p ≈ 0.012 **
STZ 60 IP	- 32.3 ± 9.8 g	- 14.6 %	**p ≈ 0.015 **
ALX 60 IP	- 41.9 ± 15.2 g	- 19.0 %	**p ≈ 0.009 **
STZ 45 IP	- 25.7 ± 11.6 g	- 11.0 %	p = 0.061 (ns)
ALX 45 IP	- 30.7 ± 14.3 g	- 13.4 %	p = 0.054 (ns)

Body weight decreased across all STZ and ALX groups over 21 days, with greater and statistically significant losses in the IV groups (especially at 60 mg/kg), while the 45 mg/kg IP groups showed smaller, non-significant weight reductions

Table 4. Interaction on body weight changes in streptozotocin and ALX treated rats

Factor	Approximate F value	p value	Interpretation
Time (Day)	≈ 28	< 0.001***	Body weight reduction over time was significant in all groups.
Group (STZ vs ALX)	≈ 9	< 0.001***	Chemical type, dose, and route of administration produced significant differences in weight change.
Time × Group interaction	≈ 6	< 0.001***	The slope of weight-loss curves over time differed among groups; IV > IP effect.

A significant main effect of time and group, together with a significant time × group interaction, indicated that body weight declined progressively in all groups but at different rates, with greater weight loss in IV-treated rats than in IP-treated rats

Table 5. Total antioxidant status (TAS) levels with streptozotocin at different doses and routes

Group	Mean TAS (mmol Trolox eq./L; mean ± SD)	Between-group p (TAS effect)	Interpretation
60 STZ IV (60 mg/kg)	0.037 ± 0.011	p = 0.832	No significant difference (i.v. vs i.p.)
60 STZ IP (60 mg/kg)	0.047 ± 0.026	p = 0.832	No significant difference (i.v. vs i.p.)
45 STZ IV (45 mg/kg)	0.585 ± 0.332	p = 0.048 *	Significant (i.p. > i.v.)
45 STZ IP (45 mg/kg)	0.933 ± 0.351	p = 0.048 *	Significant (i.p. > i.v.)
Overall ANOVA (Dose + Route)	—	p = 0.043 *	Dose and route combined effect significant

TAS levels differed according to streptozotocin dose and administration route, with no significant IV – IP difference at 60 mg/kg but significantly higher TAS in the 45 mg/kg IP group than the 45 mg/kg IV group, and an overall significant combined dose–route effect

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In the ALX-treated groups, TAS measurements differed significantly as a function of dose and administration route. In particular, the groups treated with 45 mg/kg exhibited markedly higher mean TAS levels than those receiving 60 mg/kg ($p < 0.05$). By contrast, comparing routes revealed no significant difference between i.v. and i.p. administration ($p > 0.05$). In the comparative analysis of STZ and ALX models under matched dose and route conditions, significant between-agent differences emerged. At 45 mg/kg, ALX produced markedly higher TAS values than STZ across both i.v. and i.p. administration ($p < 0.01$). At 60 mg/kg, no significant differences were observed between the two agents for either route ($p > 0.05$). Overall, ALX yielded higher TAS than STZ, with this contrast being dose-dependent and most pronounced at the lower dose (Figure 4).

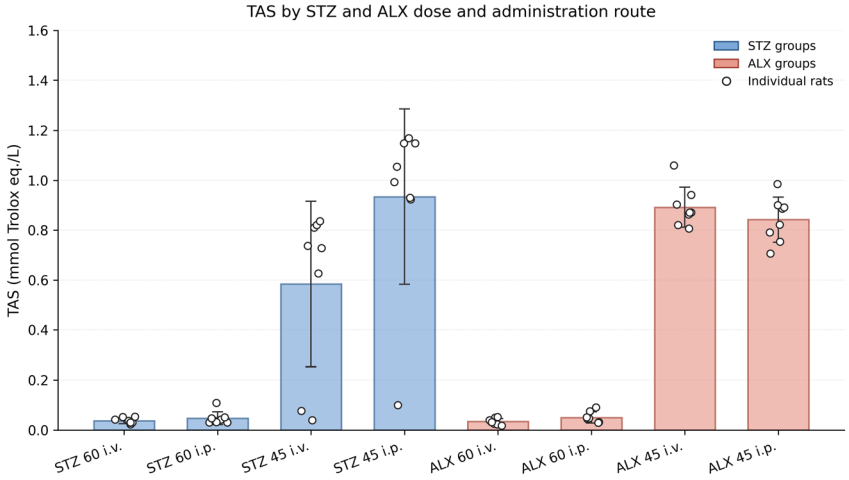

Figure 4. TAS levels in STZ and ALX groups across different doses (45, 60 mg/kg) and administration routes (i.v., i.p.); Bars represent mean ± SD; individual data points (n = 7 per group) are overlaid; Statistical comparisons used two-way ANOVA (dose × route) with Tukey post-hoc tests; *p < 0.05

When TOS values in the ALX-treated groups were examined by dose and administration route, significant differences were observed across the four groups ($p < 0.05$). The lowest mean TOS occurred in the 45 mg/kg i.v. group, although the wide dispersion in this arm (0.613 ± 0.463) meant that the pairwise differences from the remaining groups did not reach significance after Tukey adjustment ($p > 0.05$). With respect to route, mean TOS values did not differ significantly between i.v. and i.p. administration at either dose ($p > 0.05$). Across all combinations of dose (45 and 60 mg/kg) and route (i.v., i.p.), serum TOS levels were significantly higher in the ALX group than in the STZ group ($p < 0.001$). Dose did not produce significant within-agent changes in TOS for either model ($p > 0.05$) (Figure 5).

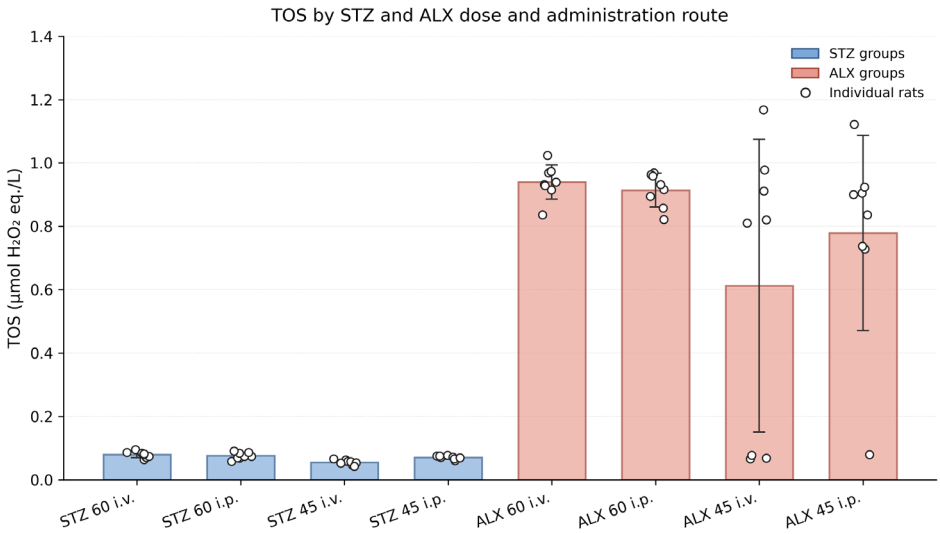

Figure 5. TOS levels in STZ and ALX groups across different doses (45, 60 mg/kg) and administration routes (i.v., i.p.); Bars represent mean ± SD; individual data points (n = 7 per group) are overlaid; Statistical comparisons used two-way ANOVA (dose × route) with Tukey post-hoc tests; *p < 0.05

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To further characterise the redox response, the OSI, defined as the ratio of TOS to TAS, was calculated for each group (Table 6). OSI displayed a marked agent- and dose-dependent pattern, with the highest values observed in the ALX 60 mg/kg arms, which substantially exceeded all STZ groups. Within the STZ arms, OSI was greater at 60 mg/kg than at 45 mg/kg, and a similar dose-dependent trend was observed in the ALX arms. Across both agents, dose appeared to be the principal determinant of systemic redox imbalance, with route exerting a secondary modulating effect. Overall, the OSI pattern reinforced the TOS and TAS findings: ALX produced a stronger oxidative burden than STZ at matched doses, and the highest oxidative load occurred under the 60 mg/kg ALX i.v. condition.

Table 6. Oxidative Stress Index (OSI) in STZ and ALX groups at different doses and administration routes

Group	TOS (μmol H_2O_2 eq./L)	TAS (mmol Trolox eq./L)	OSI
STZ 60 i.v.	0.080 ± 0.010	0.037 ± 0.011	226.6 ± 50.6
STZ 60 i.p.	0.076 ± 0.011	0.047 ± 0.026	186.2 ± 59.9
STZ 45 i.v.	0.055 ± 0.008	0.585 ± 0.332	35.4 ± 56.4
STZ 45 i.p.	0.071 ± 0.006	0.933 ± 0.351	14.8 ± 22.7
ALX 60 i.v.	0.940 ± 0.054	0.034 ± 0.012	3135.9 ± 1255.7
ALX 60 i.p.	0.914 ± 0.053	0.050 ± 0.022	2081.4 ± 705.5
ALX 45 i.v.	0.613 ± 0.463	0.892 ± 0.080	69.4 ± 53.6
ALX 45 i.p.	0.779 ± 0.308	0.842 ± 0.090	94.8 ± 41.9

Data are presented as mean ± SD; n = 7 per group; OSI was calculated as (TOS / TAS) × 100, following Erel's method [17,18]; (i.v.: intravenous; i.p.: intraperitoneal)

Discussion

This study underscores the importance of dose and route in diabetes induction with STZ and ALX in rat models. Under STZ and ALX at 45 and 60 mg/kg via different routes, fasting blood glucose exceeded 200 mg/dL in all groups after day 3, meeting the criterion for diabetes mellitus. Among the STZ arms, 60 mg/kg i.p. emerged as the most effective protocol between days 9 and 15 in terms of both magnitude and persistence of hyperglycemia, whereas 45 mg/kg i.p. sustained only a modest glycaemic elevation. These findings suggest that 60 mg/kg i.p. STZ produces a more consistent and sustained hyperglycaemic profile, whereas 45 mg/kg i.p. STZ yields an insufficient induction. Given the 21-day follow-up period, however, the present study was designed primarily to compare the efficacy of diabetes induction across agents, doses, and administration routes, rather than to evaluate long-term diabetes-related complications.

In the ALX groups, the robust glycaemic response with 60 mg/kg i.p. relative to all other dose–route combinations points to a dose-responsive β-cell toxicity. The absence of a difference between i.v. and i.p. at 45 mg/kg implies that systemic exposure at this level may minimise route-dependent effects. By contrast, 60 mg/kg i.v. produced substantially higher hyperglycemia than the 45 mg/kg groups, indicating a clear effect of dose escalation; and the fact that 60 mg/kg i.p. yielded the highest glycaemic levels suggests that the more prolonged/sustained absorption kinetics of the i.p. route may amplify β-cell injury at higher doses. Overall, for ALX, dose appears to be the primary determinant of induction strength, with route acting as a secondary modulator once a critical dose threshold is surpassed.

Notably, the 60 mg/kg ALX i.p. arm produced higher and more sustained hyperglycemia than both ALX i.v. and STZ (i.v./i.p.) from day 6 onward. This pattern suggests that ALX's β-cell toxicity is dose-sensitive and that i.p. administration sustains glycaemic elevations more persistently. ALX i.v. generated higher glucose levels than the STZ groups in the early period, but after day 15 it remained below the ALX i.p. arm. STZ i.p. yielded values exceeding STZ i.v. only on days 9 and 12. The agent effect was more pronounced with ALX than with STZ in both magnitude and duration; the highest and most persistent hyperglycemia was achieved with 60 mg/kg ALX i.p. These findings indicate that a 60 mg/kg ALX i.p. protocol produces the most consistent and sustained hyperglycaemic response among the conditions tested, whereas in STZ models route differences are more limited and confined to specific time points. In practical terms, i.v. dosing is suitable when an early glucose peak is required, while i.p. dosing is preferable when a prolonged glycaemic burden is targeted.

In a diabetic-neuropathy study that used lower doses, ALX (15 or 30 mg/kg) and STZ (17.5 or 35 mg/kg) each induced hyperglycemia. Unlike our findings, normoglycaemic animals treated with ALX or STZ showed comparable blood-glucose levels at five weeks; moreover, a large fraction remained normoglycaemic (45% with ALX 30 mg/kg; 30% with STZ), and two animals in the ALX-induced hyperglycemia group died [19]. By contrast, despite employing higher doses, we observed no mortality. The primary reason for this difference is likely variations in dosage/administration protocol and early supportive care approach; in our study, this optimization resulted in more consistent diabetes induction across all groups, while no mortality was observed.

Fajarwati et al. (2023) administered ALX (120, 150, 180 mg/kg) and STZ (40, 50, 60 mg/kg) via i.p. injection; the highest mortality occurred at 180 mg/kg ALX, the highest diabetes

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incidence at 150 mg/kg, and the greatest induction failure at 120 mg/kg [20]. In our series, diabetes was established by day 3 with 60 mg/kg ALX i.p. This discrepancy may be attributable to strain differences, as Fajarwati et al. used Sprague–Dawley rats, whereas we employed Wistar albino rats. In the same report, STZ produced the highest mortality at 60 mg/kg, the highest diabetes incidence at 50 mg/kg, and the greatest induction failure at 40 mg/kg [20]. Consistent with our data, 60 mg/kg i.p. yielded a marked glycaemic rise without mortality during the 21-day follow-up. Another study evaluating ALX and STZ delivered two injections (20 mg/kg each) via the cephalic vein at four-day intervals after 24 h fasting, with hyperglycemia maintained for four months [21]. Collectively, these observations indicate that blood-glucose outcomes vary with rat strain, age, sex, fasting duration, solution freshness and pH, injection technique, diet, and the specific model being targeted.

Body-weight loss was strongly determined by dose and route: the 60 mg/kg i.v. arm showed the greatest loss in both the ALX and STZ cohorts. In producing weight loss, i.v. exceeded i.p., and 60 mg/kg exceeded 45 mg/kg. We also found that the rate of loss differed between groups, with faster and more pronounced declines under i.v. regimens. By contrast, the modest loss in the 45 mg/kg ALX i.p. arm suggests that, given this dose–route combination, systemic exposure and/or duration may not have reached the threshold required to elicit a pronounced catabolic/weight-loss response.

STZ- and ALX-induced hyperglycemia may accelerate weight loss through osmotic diuresis/dehydration, increased lipolysis and proteolysis, and possibly reduced appetite [22]. ALX's oxidative-stress–mediated β-cell toxicity may be more prominent early, which could account for the fastest and greatest weight-loss pattern observed under high-dose i.v. administration [2]. While the i.v. route produced larger losses overall, the i.p. route—owing to more prolonged absorption—tended to maintain a steadier but smaller decline. In this framework, 60 mg/kg i.v. (especially ALX) produced the most rapid and pronounced loss, whereas 45 mg/kg i.p. yielded a more stable, lower-magnitude reduction. Assessing the magnitude of weight loss alongside glycaemic exposure (area under the curve) would aid interpretation of the weight–glycaemia relationship. In another study comparing normoglycaemic and hyperglycaemic cohorts, body-mass changes were similar in groups treated with low-dose STZ or ALX [19]. Diab et al. (2015) reported greater weight loss with STZ than with ALX when modelling diabetes using ALX 75 mg/mL and STZ 180 mg/kg, attributing the difference to STZ toxicity [23], which highlights mechanistic distinctions between STZ and ALX in pathways leading to cell necrosis [2]. Taken together, these observations are consistent with our findings for both glycaemic indices and weight loss: animals injected with either agent became diabetic and lost weight after dosing. However, in our study there was no difference in weight-loss magnitude between STZ and ALX; instead, the differences were driven by dose and route of administration.

Diabetogenic agents may precipitate oxidative stress together with hyperglycemia. The cytotoxic mechanisms of STZ and ALX are distinct. ALX generates reactive oxygen species through cyclic redox reactions [2], and the terminal products of these reactions include hydroxyl radicals that are responsible for beta cell death [24]. By inhibiting glucokinase, which serves as the glucose sensor in beta cells, ALX disrupts glucose stimulated insulin secretion. STZ exerts alkylating activity that modifies macromolecules and fragments DNA, leading to beta cell injury. Because it also fragments mitochondrial DNA, it impairs mitochondrial metabolism and thereby blunts glucose stimulated insulin secretion [2]. These mechanisms are consistent with the patterns of weight loss and blood glucose observed in our study.

When TOS and TAS were assessed together to characterise oxidative status, the profile was sensitive to both dose and route. In the ALX arms, TOS differed significantly across the four groups. The lowest TOS was recorded in the 45 mg/kg i.v. group, which was lower than the other three groups. Overall, ALX yielded higher TOS than STZ, and no significant difference in TOS was detected between i.v. and i.p. routes. Among ALX regimens, the lowest TOS occurred with 45 mg/kg i.v. For TAS, at 45 mg/kg ALX produced higher values than STZ with both i.v. and i.p. administration, whereas at 60 mg/kg there was no significant difference between agents. This pattern suggests that a compensatory antioxidant response is more prominent at the lower dose. In diabetes, however, antioxidant enzyme activities show variable results that depend on disease duration and glycaemic control, and other reports have found ALX to reduce TAS [25].

The OSI, derived as the TOS-to-TAS ratio, brought out the agent- and dose-related differences in oxidative balance more clearly than TOS or TAS considered separately. OSI was several-fold higher in the ALX 60 mg/kg groups than in any STZ group, in keeping with the cyclic redox chemistry by which ALX generates superoxide, H_2O_2 and hydroxyl radicals during its uptake by β-cells [2,4,24]. STZ, in contrast, acts predominantly through DNA alkylation and PARP activation, with oxidative stress arising as a secondary consequence of mitochondrial injury [2,6]; this difference in cytotoxic chemistry may account for the higher pro-oxidant load observed under ALX at matched doses. Within both agents, OSI was higher at 60 mg/kg than at 45 mg/kg, paralleling earlier reports that β-cell injury and the accompanying oxidative stress scale with the diabetogenic dose [6,9]. At 45 mg/kg, the lower OSI seen with ALX coexisted with higher TAS values, suggesting that a residual antioxidant capacity may be preserved at the sub-maximal dose; previous work on alloxan-diabetic rats has likewise reported that antioxidant defences vary substantially with dose and with the duration of hyperglycaemia [25,26].

Given that STZ and ALX can suppress antioxidant defences, supporting the antioxidant system may help counter oxidative stress. The literature indicates that oxidative damage to cellular components in diabetes mellitus arises from increased free radical generation accompanied by reduced antioxidant capacity

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[26], and a decrease in antioxidant levels may contribute to hyperglycemia. In our dataset, lower dose STZ and ALX were associated with higher antioxidant capacity regardless of route. In diabetes, macrophage infiltration into adipose tissue increases the release of inflammatory cytokines and raises ROS production, and the present work helps to outline one of the mechanistic pathways underlying the diabetic state [27].

This study has several limitations. It was conducted at a single centre and included only male Wistar rats within a restricted two-dose/two-route framework (45/60 mg/kg; i.v./i.p.); therefore, other strains, female animals, and a broader range of doses and administration routes were not evaluated. The 21-day follow-up period did not allow detailed assessment of long-term complications, mortality, or reversibility. More fundamentally, the present study was designed primarily as a comparative dose–route screening to identify which agent, dose, and administration route combinations reliably elicit and sustain hyperglycaemia under matched conditions. For this purpose, glycaemia, body weight, and systemic redox balance (TOS/TAS) were selected as practical and easily transferable readouts. Accordingly, direct markers of β-cell injury — including fasting insulin, C-peptide, HbA1c, glucose tolerance, lipid profile, and pancreatic histopathology with islet morphology and β-cell mass — were beyond the scope of the present design. Incorporating these parameters in subsequent studies will be essential to confirm and extend the present findings at the molecular and tissue levels. In addition, because food and water intake, ketonaemia/ketonuria, and hydration status were not monitored, the mechanisms underlying weight loss could not be fully clarified. Therefore, the generalisability of the present findings should be confirmed in future studies with longer follow-up, inclusion of both sexes and different strains, a broader biomarker panel combined with histopathological evaluation, and preferably multi-centre designs.

These findings may provide practical guidance for researchers in selecting the most appropriate diabetogenic agent, dose, and route of administration for establishing reliable and reproducible experimental diabetes models in preclinical studies.

Conclusion

In summary, our data in male Wistar rats show that both dose and route decisively shape the glycaemic, weight-loss, and redox responses to STZ and ALX. All groups exceeded 200 mg/dL by day 3, confirming diabetes; among the STZ arms, 60 mg/kg i.p. yielded the highest hyperglycemia between days 9 and 15, whereas 45 mg/kg i.p. remained substantially lower throughout. For ALX, dose was the primary determinant: 60 mg/kg i.p. produced the highest and most sustained hyperglycemia from day 6. When TOS and TAS were considered together, the highest oxidative burden occurred with 60 mg/kg ALX, and the lowest with 45 mg/kg i.v. Body-weight loss was greatest with 60 mg/kg i.v. STZ or ALX. Taken collectively, these findings indicate that 60 mg/kg ALX and 60 mg/kg STZ i.p. are suitable starting options for diabetes induction based on glycaemic and redox endpoints, with route effects more pronounced for ALX than for STZ. Confirmation of model validity at the tissue and molecular level will require complementary studies incorporating histopathological and metabolic markers. Given the single-centre design, 21-day follow-up, and a limited biomarker panel, future work with longer follow-up and expanded biochemical/histological endpoints is warranted to validate and generalise these results.

Ethical Approval

All procedures were reviewed and approved by the Ankara Medipol University Animal Experiments Local Ethics Committee (HADYEK) (03 December 2025; Protocol No. AMUHADYEK-24; Document No. E-21265738-20.01-11797) and were conducted in accordance with national and institutional guidelines for the care and use of laboratory animals.

Patient Consent

Not applicable. This study was performed exclusively on male Wistar albino rats and did not involve human participants, human data, human tissue or identifiable patient information; therefore, patient consent was not required. Ethical approval details are given in the Ethical Approval section above.

Conflict of Interest

The authors confirm the absence of any conflicts associated with this work.

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