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Comparative study of diabetes induction in rabbits and rats using streptozotocin: a pilot study

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The study was aimed to induce diabetes mellitus in both Wistar albino rats and New Zealand white rabbits using Streptozotocin (STZ) to assess their suitability as models for diabetes research. Eight male rats weighing 190-240 g and eight male rabbits weighing 1.5-2 kg were administered STZ intraperitoneally at varying doses: 40 mg/kg body wt., 42 mg/kg body wt., 45 mg/kg body wt., and 50 mg/kg body wt. in rats, and 45 mg/kg body wt., 50 mg/kg body wt., 55 mg/kg body wt., and 60 mg/kg body wt. in rabbits. Blood glucose levels were measured 72 hr post-administration to confirm the induction of hyperglycemia. The animals with blood glucose levels exceeding 250 mg/dL were classified as diabetic. Body weights were recorded on days 3, 7, 14, and 21 to monitor changes associated with diabetes. Rats receiving 45 mg/kg bodt wt. and 50 mg/kg body wt. STZ succumbed within 14 and 7 days, respectively (pancreas were collected for histopathology), while those administered 42 mg/kg body wt. maintained blood glucose levels above 270 mg/dL for the entire 21-day study period. In contrast, rats given 40 mg/kg body wt. did not sustain elevated blood glucose levels beyond 14 days. A marked reduction in body weight was observed in diabetic rats after day 3. However, none of the rabbits developed hyperglycemia at the administered doses, and there was no significant variation in body weight observed. All animals were euthanized on day 21, and pancreatic tissues were collected for histopathological analysis. The findings suggest that STZ at 42 mg/kg body wt. can reliably induce diabetes in rats, whereas the same is not achievable in rabbits at the doses tested. Therefore, rats serve as a suitable model for diabetes studies, while rabbits do not appear to be viable for this purpose using STZ.

Key words: *Diabetes mellitus, Pancreas, Rabbit, Rat, Streptozotocin*

Diabetes mellitus is marked by elevated blood sugar levels (hyperglycemia) due to defects in insulin secretion, its action, or a combination of both (Kottaisamy *et al*., 2021). No single animal model can entirely replicate the full spectrum of the diabetic disease process and its diverse manifestations, as each model only captures a specific facet of this complex condition (Grada *et al*., 2018). Chemically induced diabetes mellitus (DM) models are relatively more affordable and simpler to create and manage compared to many other diabetic animal models (Srinivasan and Ramarao, 2007). Chemically induced animal models of diabetes mellitus (DM) are commonly utilized in various research areas, though one major drawback is the difficulty in maintaining survival rates after induction (Bacevic *et al*., 2020). Streptozotocin and alloxan are the most commonly used drugs to induce pancreatic islet necrosis, leading to the development of diabetes mellitus (Bacevic *et al*., 2020). Streptozotocin (STZ) is an antibiotic that induces the destruction of pancreatic islet â-cells and is commonly used in experimental settings to create a model of type 1 diabetes mellitus (T1DM) (Furman, 2015). Diabetes can be chemically induced in mice and rats through intraperitoneal or caudal vein injections of streptozotocin or alloxan, which selectively destroy the insulin-producing beta cells in the pancreas (Grada *et al*., 2018). Rats are frequently used as models for STZ-induced diabetes (Furman, 2015). The aim of the present study was comparative evaluation of diabetes induction in rabbits and rats using streptozotocin

Materials and Methods

The study was conducted on eight male Wistar albino rats weighing 190-240 g and eight male rabbits weighing 1.5-2 kg after obtaining permission from institutional animal ethics committee vide proposal number IAEC/06.10.2023/S4. The rats were randomly divided into 4 groups with 2 animals in each group i.e., group A, B, C, and D. Streptozotocin (Sisco Research Laboratories Pvt. Ltd., Mumbai, India) was administered @ 40 mg/kg body wt. (group A), 42 mg/kg body wt. (group B), 45 mg/kg body wt. (group C), and 50 mg/kg body wt. (group D) in all the four groups via intraperitoneal route (Fig.1). The STZ solution was freshly prepared right before injection and administered within 5 min of dissolving. Similarly the rabbits were randomly divided into 4 groups with 2 animals in each group i.e., group E, F, G, and H. Streptozotocin was administered @ 45 mg/kg body wt. (group E), 50 mg/kg body wt. (group F), 55 mg/kg body wt. (group G), and 60 mg/kg body wt. (group H)

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in all the four groups. To prevent fatalities from hypoglycemic shock following induction, a 5% glucose solution was provided as drinking water for up to 24 hr after STZ administration. The body weight and fasting blood glucose (FBG) values were estimated on days 3, 7, 14, and 21 post STZ administrations. All animals were euthanized on day 21, and pancreatic tissues were collected for histopathological analysis including diabetic rats that succumbed on day 7 and 14 (Fig. 2).

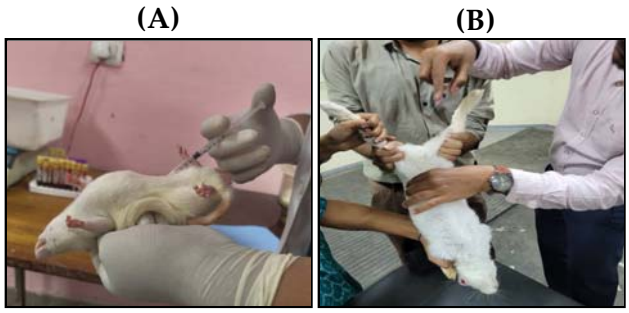

Fig. 1: Induction of diabetes in (A) rat and (B) rabbit using intraperitoneal administration of STZ.

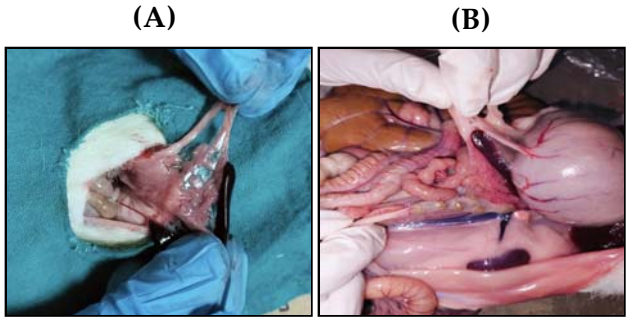

Fig. 2: Gross image of pancreas (arrow) in A) rat and B) rabbit.

Statistical analysis was conducted using GraphPad Prism software (version 8). The mean±SE of different parameters were compared using two-way ANOVA followed by Tukeys multiple comparison test.

Results and Discussion

On general clinical examination, the rats in group A remained active and alert, while those in groups B, C, and D appeared dull and depressed starting from day 3 post-STZ administration, with these signs becoming more pronounced after day 7, as most rats exhibited a lean appearance due to fat loss. Rats in groups C and D succumbed after day 14 and 7, respectively, due to hyperglycemia and complications of diabetes. The mortality could be attributed to the toxic STZ dosages (Furman, 2015). Literature indicates that various doses of STZ, starting as low as 40 mg/kg body wt. and higher (70 mg/kg), have been used to induce diabetes mellitus, with toxicity generally increasing in proportion to the dose (Brøndum *et al*., 2005). Since female mice are less susceptible to the toxic effects of this islet-cell toxin, the majority of STZ-induced diabetes studies are performed on male animals (Furman, 2015). Inducing experimental diabetes in rats through chemicals that specifically target and destroy pancreatic B cells is both convenient and straightforward (Szkudelski, 2001). Polyuria and polydipsia were noticed in diabetic rats after STZ administration. In contrast, all rabbits in groups E, F, G, and H were active and alert throughout the study period, showing an increase in body weight compared to day 0 and maintained normal appetite. Both polyuria and proteinuria were indicative of diabetes (Wang *et al*., 2010), which were very evident in the diabetic rats. The reported mortality rates vary significantly based on factors such as the animal species, dosage, chemicals administered, and protocols followed (Bacevic *et al*., 2020).

Table 1: The body weight (g) in different groups of rats on days 0, 3, 7, 14, and 21.

	Day 0	Day 3	Day 7	Day 14	Day 21
Group A (40 mg/kg body wt.)	232.5 ±2.5	226.5 ±1.5[a]	223 ±3[a]	226.5 ±5.5[a]	232.5 ±1.5
Group B (42 mg/kg body wt.)	223.5 ±5.5	209 ±8[b]	199.5 ±2.5[b]	181.5 ±0.5[b]	170.5 ±4.5
Group C (45 mg/kg body wt.)	226±14	218±10[b]	175±5[b]	172±4[b]	-
Group D (50 mg/kg body wt.)	224.5 ±3.5	177.5 ±2.5[b]	172.5 ±2.5[b]	-	-

Different superscripts indicate significant differences among groups (P<0.05)

Table 2: The body weight (kg) in different groups of rabbits on days 0, 3, 7, 14, and 21.

	Day 0	Day 3	Day 7	Day 14	Day 21
Group E (45 mg/kg body wt.)	1.6 ±0.04	1.61 ±0.04	1.62 ±0.07	1.65 ±0.06	1.68 ±0.05
Group F (50 mg/kg body wt.)	1.63 ±0.04	1.63 ±0.05	1.65 ±0.04	1.69 ±0.01	1.7 ±0.01
Group G (55 mg/kg body wt.)	1.65 ±0.15	1.69 ±0.16	1.71 ±0.15	1.76 ±0.18	1.78 ±0.17
Group H (60 mg/kg body wt.)	1.79 ±1.79	1.82 ±1.82	1.84 ±1.84	1.89 ±1.89	1.94 ±1.94

Different superscripts indicate significant differences among groups (P<0.05)

In the case of rats, the body weight of group A animals did not show a significant difference (P>0.05)

from day 0 (before STZ administration) (Table 1 and 2). In groups B, C, and D, although a drastic reduction in body weight was observed, making the rats appear noticeably lean, this difference was not statistically significant. The comparison of body weight among the rat groups revealed that the values in groups B, C, and D were significantly lower than those in group A on days 3, 7, and 14. In rabbits, there is a tendency for weight gain following diabetes induction contrary to rats (Wang *et al*., 2010). In rabbits, there was a non-significant (P>0.05) increase in body weight following STZ administration at the specified doses across the groups (E, F, G, and H). This mighty be attributed to the post-administration care provided. However, there was no significant difference in body weight among different groups (E, F, G, and H) of rabbits.

Table 3: The fasting blood glucose levels (mg/dL) in different groups of rats on days 0, 3, 7, 14, and 21.

	Day 0	Day 3	Day 7	Day 14	Day 21
Group A (40 mg/kg body wt.)	104±8	297 ±15^{a}	266.5 ±5.5^{a}	259.5 ±1.5^{a}	113±1
Group B (42 mg/kg body wt.)	109.5 ±0.5	313.5 ±21.5^{b}	325 ±19^{a}	375 ±24^{a}	404±11*
Group C (45 mg/kg body wt.)	111.5 ±3.5	407±8^{*b}	464 ±7^{*b}	556.5 ±3.5^{*b}	-
Group D (50 mg/kg body wt.)	99.5 ±1.5	475.5 ±16.5^{*b}	545.5 ±31.5^{*b}	-	-

*Values with * are significantly different from day 0 (baseline) (P<0.05)*

Different superscripts indicate significant differences among groups (P<0.05)

Table 4: The fasting blood glucose levels (mg/dL) in different groups of rabbits on days 0, 3, 7, 14, and 21.

	Day 0	Day 3	Day 7	Day 14	Day 21
Group E (45 mg/kg body wt.)	113.5 ±2.5	117.5 ±1.5	112.5 ±0.5	96.5 ±4.5	106.5 ±4.5
Group F (50 mg/kg body wt.)	109.5 ±0.5	112 ±1	114.7 ±2.3	108±1	110.5 ±1.5
Group G (55 mg/kg body wt.)	117.5 ±2.5	109 ±12	109 ±10	103.5 ±1.5	105±7
Group H (60 mg/kg body wt.)	109.5 ±11.5	102 ±27	104.5 ±15.5	105.5 ±13.5	106.5 ±11.5

In group A rats, fasting blood glucose (FBG) levels increased slightly but not significantly (P>0.05) from baseline up to day 7, followed by a gradual decrease, returning to normal by day 21 (Table 3 and 4). In group B rats, hyperglycemia persisted until day 21, with significantly (P<0.05) elevated FBG levels observed on day 21. Similar findings were reported by Furman (2015), wherein 50% of the rats were severely hyperglycemic by day 21. In groups C and D, pronounced hyperglycemia was detected as early as day 3, and the elevated FBG levels were sustained until their death on days 14 and 7, respectively. Fasting blood glucose levels for mild hyperglycemia should exceed 270 mg/dL and/or higher in STZ-injected rats compared to control rats (Furman, 2015). The comparison of blood glucose levels between the rat groups showed that the FBG values in groups B, C, and D were significantly higher than those in group A on days 3, 7, and 14. In contrast, none of the rabbits in groups E, F, G, and H exhibited hyperglycemia after STZ administration. The normal blood glucose level in rabbits ranges from 102 to 145 mg/dL. Thus, all the rabbits maintained normal blood glucose levels after STZ administration, indicating the absence of diabetes. There was no significant difference between different groups of rabbits (P>0.05).

The histophotomicrograph of the rat pancreas in

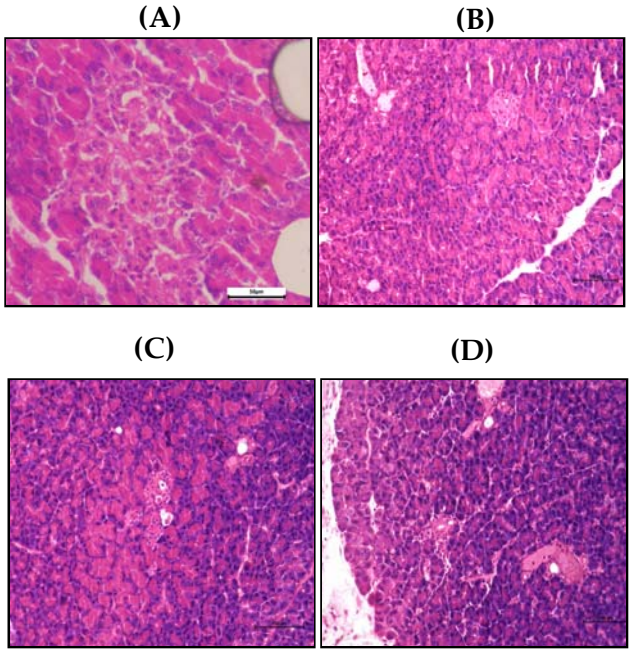

Fig. 3: Histophotomicrograph of the rat pancreas: (A) group A showed normal-sized islets of Langerhans (star) displaying localized degeneration of â-cells in the centre and intact acinar cells (arrow head), H&E; x200. (B) group B, (C) group C, and (D) group D exhibited significant alterations in both the exocrine and endocrine components, with swollen acinar cells with small vacuoles were observed throughout most areas, and the interlobular ducts were lined with flattened epithelial cells (arrow head). The islets of Langerhans displayed shrinkage, degeneration, and necrosis of component cells (star). The nuclei appeared densely basophilic with clear evidence of karyolysis. Streptozotocin (STZ) caused severe degeneration of â-cells, leading to an almost complete loss of islet â-cells in STZ-treated rats, H&E; x 200).

group A revealed normal-sized islets of Langerhans, showing regenerative changes, particularly in the â cells located centrally **(**Fig. 3 and 4**)**. However, the pancreas from groups B, C, and D displayed marked alterations due to diabetes in both the exocrine and endocrine components. Notable changes included swollen acinar cells containing small vacuoles across most regions, with interlobular ducts lined by flattened epithelial cells. The islets of Langerhans exhibited shrinkage, degeneration, and necrosis of constituent cells, with nuclei appearing densely basophilic and showing clear signs of karyolysis. In Streptozotocin (STZ)-treated rats, severe â-cell degeneration occurred, leading to an almost complete depletion of islet â-cells. The pancreas of diabetic rats showed shrinkage of the islets of Langerhans, with degeneration and necrosis of the component cells, whose nuclei appeared densely basophilic and exhibited signs of karyolysis (Saad *et al*., 2015; Safitri *et al*., 2021).

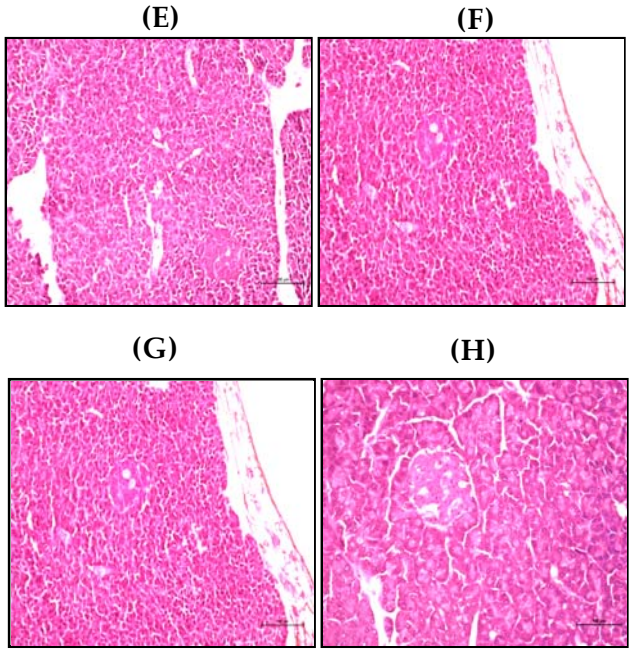

Fig. 4: Histophotomicrograph of the rabbit pancreas showing normal architecture (H&E; x 200): (E) group E, (F) group F, (G) group G, and (H) group H. The exocrine portion consists of densely packed acinar cells, arranged in small lobules and separated by distinct intralobular and interlobular connective tissue septa (arrow head). The islets of Langerhans are scattered among the acinar cells, appearing lighter than the surrounding acinar tissue (star).

In contrast, the histophotomicrographs of the rabbit pancreas in groups E, F, G, and H demonstrated a largely preserved architecture. The exocrine portion composed of densely packed acinar cells, organized into small lobules separated by distinct intralobular and interlobular connective tissue septa. The islets of Langerhans, scattered among the acinar cells, appeared lighter in staining compared to the surrounding acinar tissue, indicating intact endocrine structures. The histopathological examination of the pancreas in diabetic rabbits reveals significant beta-cell damage and thickened arterial walls (Wang *et al*., 2010); however, such findings were not observed in the current study.

Based on the findings of this study, it is evident that while STZ is an effective agent for inducing diabetes in rats, but it is not suitable for inducing diabetes in rabbits. Streptozotocin and alloxan are widely used to induce experimental diabetes mellitus (DM) in animals such as mice, rats, rabbits, and dogs. While streptozotocin is the preferred agent for inducing DM in rodents, alloxan is regarded as the optimal choice for rabbits (Wang *et al*., 2010; Mir *et al*., 2013; Luo *et al*., 2024). Most studies related to diabetes in rabbits are conducted using alloxan, indicating it as the preferred drug for inducing diabetes (Luo *et al*., 2024; Wang *et al*., 2010). Although a few studies report the effectiveness of streptozotocin (STZ) in inducing diabetes at the doses used in our research (Mir *et al*., 2015), our study demonstrates that STZ is ineffective in producing diabetes in rabbits. STZ administered to rabbits at 65 mg/kg body wt. as a single intravenous dose caused immediate hyperglycemia, followed by sustained hypoglycemia requiring glucose therapy within 9 hr. However, this study does not support the use of rabbits as a suitable diabetic model (Mir *et al*., 2015). Rabbits and guinea pigs are resistant to streptozotocin's diabetogenic effects, likely due to their use of an alternate pathway for NAD synthesis from nicotinic acid, which the drug does not block (Kushner *et al*., 1969). This maintains the comparison and emphasizes STZ's limited efficacy in rabbits compared to rats.

The study highlights distinct physiological responses to Streptozotocin (STZ) administration between rats and rabbits. Rats in groups B, C, and D developed clinical signs of diabetes, including hyperglycemia, weight loss, polyuria, and polydipsia, following STZ induction. These effects were accompanied by histological evidence of pancreatic â-cell destruction, with groups C and D succumbing to hyperglycemia and complications associated with diabetes. The findings align with existing literature, indicating that STZ induces diabetes in a dose-dependent manner, particularly in male rats, and leads to significant metabolic disturbances. However, in rabbits, STZ administration did not result in hyperglycemia or significant pancreatic damage across all groups. The rabbits maintained normal fasting blood glucose levels and even showed a slight increase in body weight during the study period. Histological examination of the rabbit pancreas revealed a largely intact architecture, without evidence of â-cell destruction or other significant diabetic pathology. This suggests that rabbits are more resistant to the diabetogenic effects of STZ compared to rats, likely due to species-specific

differences in sensitivity to this toxin (Kushner *et al*., 1969; Lazar *et al*., 1968). The literature supports this observation, noting that rabbits, like other species such as female rodents, are less susceptible to STZ-induced pancreatic â-cell damage. This species-specific resistance may limit the use of STZ in rabbit models for diabetes research, as the standard doses that reliably induce diabetes in rodents fail to produce similar effects in rabbits.

The study of this study indicated that streptozotocin (STZ) was effective in inducing diabetes in rats, with a dose of 42 mg/kg body wt. successfully producing sustained hyperglycemia throughout the study period. Doses below 42 mg/kg body wt. failed to maintain hyperglycemia consistently, while doses above 42 mg/kg body wt. proved fatal. However, in the case of rabbits, STZ was ineffective at all four doses tested, indicating that it was not a suitable drug for inducing diabetes in rabbits based on gross, biochemical and histopathological examination.

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