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| RESEARCH ARTICLE

Impact of Low and High Dose of Streptozotocin on the Levels of Antioxidants and Liver Enzymes in Rats

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| ABSTRACT

Background: Streptozotocin (STZ) is the most prominent diabetogenic chemical that is widely used in experimental animals for creating animal models of diabetes. Obtaining valid data from STZ-based animal models of diabetes depends on the correct preparation and use of STZ. **Aims**: The current study aims to investigate the impact of streptozotocin injected at different doses on the levels of antioxidants and liver enzymes in rats. **Materials and methods**: A total of 35 female rats were purchased, acclimated, and divided randomly and equally into 5 groups; NCG (as non-injected-negative control), EG1 (streptozotocin-injected at 50mg/kg.BW), EG2 (streptozotocin-injected at 100mg/kg.BW), EG3 (streptozotocin-injected at 150mg/kg.BW) and EG4 (streptozotocin-injected at 200mg/kg.BW). After 72 hours of streptozotocin injection, blood samples were collected directly from heart, and the obtained sera were tested quantitatively by ELISA to measurement the concentrations of antioxidants (catalase, glutathione peroxidase [GPX], and superoxide dismutase [SOD]) as well as the liver enzymes (alkaline phosphatase [ALP], alanine aminotransferase [ALT], and aspartate aminotransferase [AST]). **Results**: In comparison to values of NCG, the findings of all experimentally groups (EG1, EG2, EG3, and EG4) were shown a significant decrease in values antioxidants (catalase, GPX, and SOD) and a significant elevation in values of liver enzymes (ALP, ALT, and AST). Among the experimentally study groups, the highest values of antioxidants were detected in EG1 while the lowest was seen in EG4. In contrast, the lowest value of liver enzymes was observed in EG1 and the highest in EG4. **Conclusion**: This study revealed the negative effect of streptozotocin on targeted biomarkers, suggesting its role in the development of oxidative stress and related complications in a number of body tissues. Subsequently, the impact is dose-dependent since the high dose of streptozotocin causes severe alteration in the values of all antioxidants and liver enzymes. However, additional studies are of great importance to estimate the effect of streptozotocin on different enzymes, hormones and body markers even tissues and organs.

| KEYWORDS

Diabetogenic agents, Animal models, *Streptomyces achromogenes*, pancreatic islets, Iraq

| ARTICLE INFORMATION

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1. Introduction

Streptozotocin is a naturally occurring methylnitrosourea antineoplastic antibiotic compound. It was isolated from the bacterium *Streptomyces achromogenes* by scientists working at the drug company Upjohn (now part of Pfizer) in the late 1950s (Majeed et al., 2008; Zhu, 2022). During the mid-1960s, studies conducted on streptozotocin found that it is selectively toxic to the β cells of the pancreatic islets (Sithole, 2009). This finding led to the use of streptozotocin in an animal model of diabetes (Goyal et al., 2016). Streptozotocin was discovered as an anticancer agent serendipitously in 1965 when Evans and his colleagues found its antitumor effects (Poduri, 2021). Later,

Murray-Lyon et al. (1971) used this agent in controlling intractable hypoglycemia in a patient with metastatic carcinoma of the pancreatic islets. They found excellent symptomatic relief and hepatic metastases reduced in size in the patient with a malignant islet-cell tumor of the pancreas treated with streptozotocin. In the 1960s and 1970s, the NCI investigated the use of streptozotocin in cancer chemotherapy. In addition, streptozotocin and its analogs have been found to be effective against several other cancer types, such as leukemia (Aoki et al., 2015; La Salvia et al., 2024). In Phase II clinical trials, streptozotocin has also been used in combination with other drugs including procarbazine, 6-thioguanine (Horton et al., 1975), and CCNU for the treatment of metastatic colon cancer, and in combination with bleomycin, CCNU (Levi et al., 1977), tilorone, Baker's antifol, 5-fluorodeoxyuridine, and arabinosyl cytosine for breast cancer (Cummings et al., 1981). Thus, streptozotocin is being used for the treatment of several types of cancer alone or in combination with other therapeutic drugs (Bollard et al., 2018).

Models are needed when we cannot put our hands on the object of the study. An animal model is a living organism in which a phenomenon of interest, similar in some aspects to humans, is studied in a way that cannot be studied in humans (Hau, 2008). Using animal models in biomedical research has a long history (Franco, 2013). An estimate in 2005 indicated that the number of laboratory animals used for research was about 115 million per year and is still increasing (Akhtar, 2015). A report on toxicological studies indicates that the concordance between adverse findings in clinical data and data produced in experimental animals was 71% (Ewart et al., 2014). In addition, according to both the Nuremberg Code and the Declaration of Helsinki, which are cornerstones for conducting ethical biomedical research, animal studies need to be conducted before human trials (Hall and Traystman, 2009; de Vries et al., 2014; Faggion, 2015). However, there is controversy concerning the predictive power of animal models (Shanks et al., 2009). Results obtained from animals are not predictive of human response and should be only used for generating hypotheses to be tested in humans (Uhl and Warner, 2015). A high rate of new drugs that passed preclinical studies fail in the clinical phase, such as the average successful translation rate from animals to clinical cancer trials is <8% (Mak et al., 2014; He et al., 2019). Thus, animal models are excellent basic science tools but are not appropriate for biomedical prediction (Vashishat et al., 2024). The results of animal studies can be used for building conceptual models to generate testable hypotheses to verify in humans (Wall and Shani, 2008). In other words, animal models of human diseases provide an understanding of the studied disease and are not intended to act as a direct one-to-one surrogate (Swearengen, 2018). Despite the limitations mentioned, animal models have remained the best alternative way for testing hypotheses before human trials and are a core of preclinical drug development, which is a lengthy and expensive process (Garattini and Grignaschi, 2017; Singh and Seed, 2021). An ideal animal model should mimic natural disease patterns in humans as closely as possible; however, none of the models corresponds to human disease, and each model provides advantages for studying some areas of the disease (McGonigle and Ruggeri, 2014; Mukherjee et al., 2022; Loewa et al., 2023). The current study aims to investigate the impact of streptozotocin when given at single different doses (200mg/kg.BW, 150mg/kg.BW, 100mg/kg.BW, and 50mg/kg.BW) on the levels of antioxidants (catalase, GPX, and SOD) and liver enzymes (ALT, ALP, and AST) in rats using the quantitative enzyme-linked immunosorbent assay (ELISA).

2. Materials and methods

2.1 Ethical approval

This study was licensed by the Scientific Committee of the College of Veterinary Medicine (University of Wasit, Wasit, Iraq).

2.2 Preparation of study animals

In total, 35 female rats of 118 – 159 grams of weight were purchased from the private animal house in Baghdad province (Iraq) and transported to the Animal House in the College of Veterinary Medicine at the University of Wasit. Initially, the study animals were subjected to a preparation period of 1 week, during which they were fed a pellet, presented to tap water, and exposed to 12 / 12 hours of light/dark.

2.3 Study designing

Randomly, the study rats were divided equally into 5 groups as follows:

1. Negative control group (NCG): Rats of this group were not injected with streptozotocin and were fed normally.
2. Experimental group 1 (EG1): Rats of this group were subjected to a single intraperitoneal injection of streptozotocin at a dose of 50mg/kg.BW, and feed normally (Arunachalam and Parimelazhagan, 2013).
3. Experimental group 2 (EG2): Rats of this group were subjected to a single intraperitoneal injection of streptozotocin at a dose of 100mg/kg.BW, and feed normally (Arunachalam and Parimelazhagan, 2013).
4. Experimental group 3 (EG3): Rats of this group were subjected to a single intraperitoneal injection of streptozotocin at a dose of 150mg/kg.BW, and feed normally (Arunachalam and Parimelazhagan, 2013).
5. Experimental group 4 (EG4): Rats of this group were subjected to a single intraperitoneal injection of streptozotocin at a dose of 200mg/kg.BW, and feed normally (Furman, 2015).

2.4 Samples

After 72 hours of streptozotocin injection, all study animals were anesthetized with chloroform and subjected to direct blood draining from the heart using a disposable syringe. The blood samples were collected into labeled free-anticoagulant glass gel tubes that were kept vertically at room temperature for 30 min, centrifuged at 5000 rpm for 15 minutes, and the sera of each sample were collected into a labeled Eppendorf tube and kept frozen in the refrigerator until be used for measurement of antioxidants and liver enzymes (Wahab et al., 2024; Al-Khatawi et al., 2025).

2.5 Biochemical measurement of antioxidants and liver enzymes

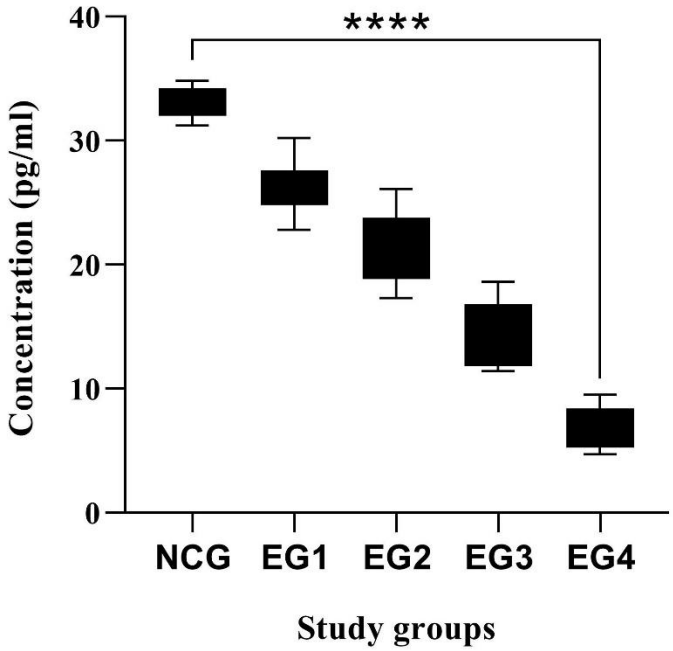
According to manufacturer instructions for quantitative ELISA kits (SunLong Biotech, China), the serum samples and the contents of each kit (Standard, Diluent, and Solution) were prepared and processed at room temperature. After the addition of the stop solution, the optical density (OD) was read at an absorbance of 450nm using the Microplate ELISA Reader. Then, the ODs and concentrations of Standards, in addition to the ODs of samples, were plotted on a log scale to calculate the concentration of each biomarker.

2.6 Statistical analysis

One-Way Analysis of Variance (ANOVA) in the GraphPad Prism Software was used to detect significant differences between the obtained values of the five study groups at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****). Values of study results were represented as Mean ± Standard Error (M±SE) (Gharban, 2024).

3. Results

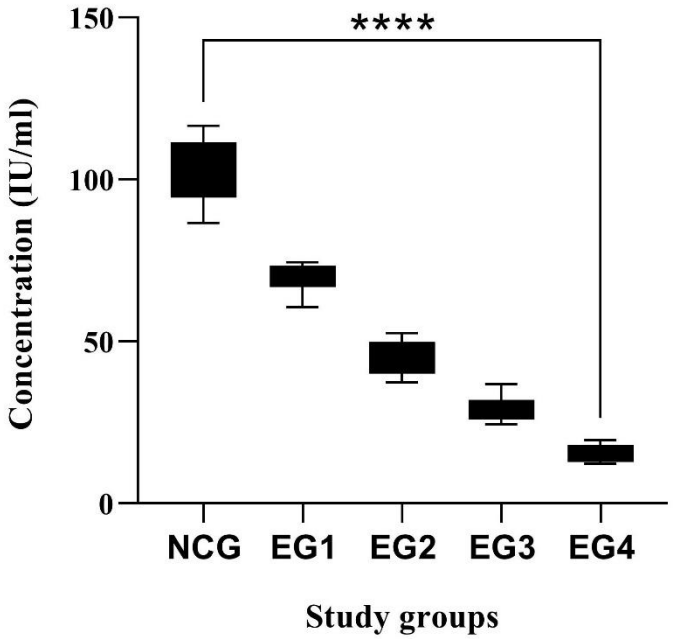
In relation to values of NCG (32.975 ± 0.432 pg/ml), the findings of catalase were decreased significantly ($p<0.0001$) among the experimental groups; EG1 (26.525 ± 0.791), EG2 (21.838 ± 1.045 pg/ml), EG3 (14.757 ± 1 pg/ml), and EG4 (6.74 ± 0.809 pg/ml). Significantly ($p<0.05$), the highest value among the experimental groups was detected in EG1, while the lowest was shown in EG4 (Figure 1).



Group	M ± SE
NCG	32.975 ± 0.432
EG1	26.525 ± 0.791
EG2	21.838 ± 1.045
EG3	14.757 ± 1
EG4	6.74 ± 0.809
p-value	0.0001 ****
95% *CI*	7.904 to 33.23
R^2	0.9426

Figure (1): Concentrations of catalase among the rats of five study groups

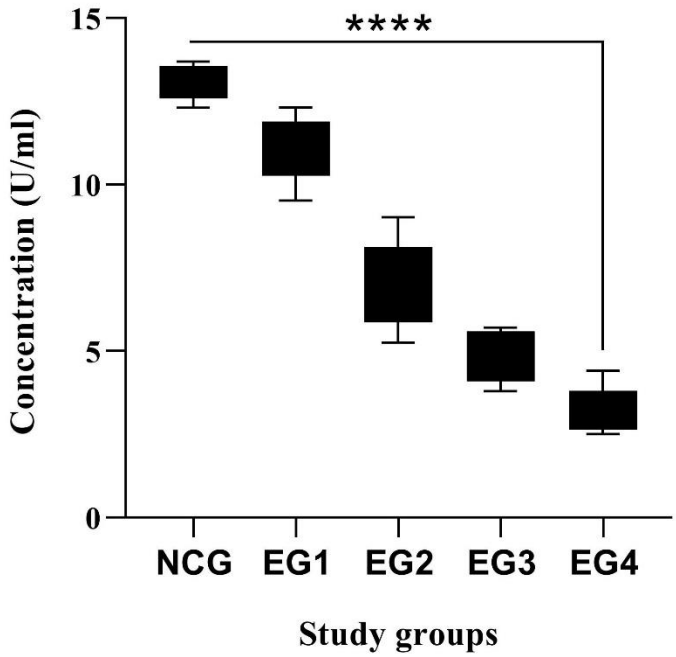
Significantly, the findings of GPX were reduced ($p<0.0001$) in experimental groups: EG1 (69.588 ± 1.603 IU/ml), EG2 (45.313 ± 1.889 IU/ml), EG3 (29.614 ± 1.55 IU/ml), and EG4 (15.1 ± 1.315 IU/ml) when compared to those of NCG (103.388 ± 3.577 IU/ml). Among the experimental study groups, the highest value of GPX was seen significantly ($p<0.05$) in EG1, while the lowest was observed in EG4 (Figure 2).



Group	M ± SE
NCG	103.388 ± 3.577
EG1	69.588 ± 1.603
EG2	45.313 ± 1.889
EG3	29.614 ± 1.55
EG4	15.1 ± 1.315
p-value	0.0001 ****
95% *CI*	9.339 to 95.86
R^2	0.9656

Figure (2): Concentrations of GPX among the rats of five study groups

Regarding the concentrations of SOD, there was a significant reduction ($p<0.0001$) in values of all experimental groups; EG1 (11.026 ± 0.344 U/ml), EG2 (7.079 ± 0.457 U/ml), EG3 (4.7 ± 0.278 U/ml), and EG4 (3.16 ± 0.33 U/ml) when compared to result of NCG (13.15 ± 0.185 U/ml). Subsequently, the findings of EG4 were significantly ($p<0.05$) lower than those of other experimental study groups: EG1, EG2 and EG3 (Figure 3).

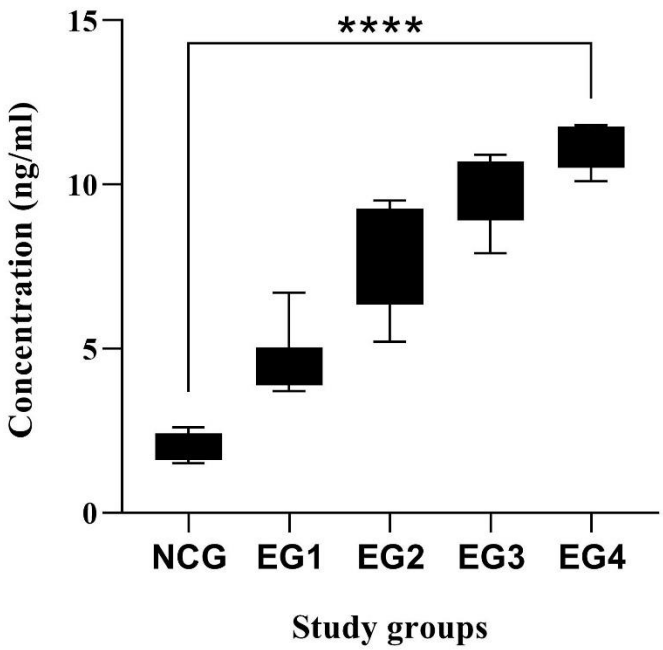


Group	M ± SE
NCG	13.15 ± 0.185
EG1	11.026 ± 0.344
EG2	7.079 ± 0.457
EG3	4.7 ± 0.278
EG4	3.16 ± 0.33
p-value	0.0001 ****
95% *CI*	2.603 to 13.04
R^2	0.9495

Figure (3): Concentrations of SOD among the rats of five study groups

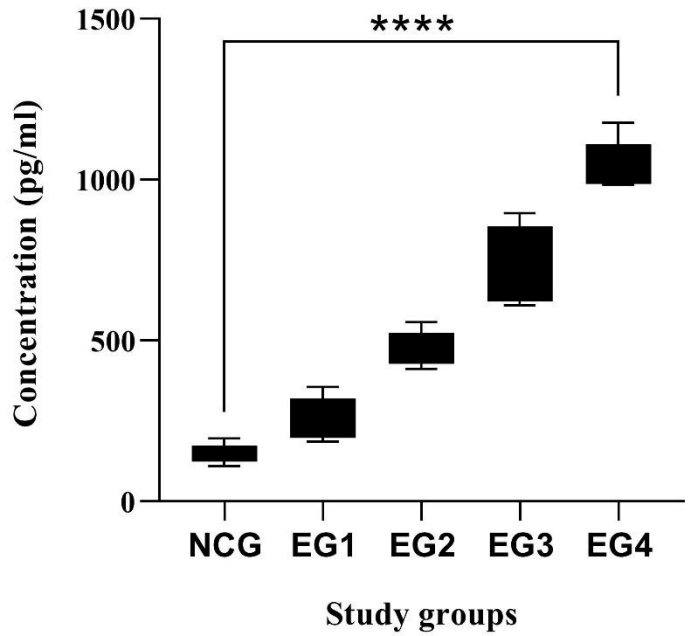
Among the values of liver enzymes, significant differences ($p<0.05$) were reported in values of various study groups. For ALP, significant elevation ($p<0.0001$) in values of experimental groups: EG1 (4.65 ± 0.341 ng/ml), EG2 (7.65 ± 0.555 ng/ml), EG3 (9.714 ± 0.408 ng/ml), and EG4 (11.18 ± 0.312 ng/ml) was shown in comparison to results of NCG (2.025 ± 0.149 ng/ml). Significantly ($p<0.05$), the highest value among experimentally studied groups was seen in EG4, whereas the lowest was reported in EG1 (Figure 4).

The concentration of ALT in the current study showed a significant elevation ($p<0.0001$) among experimentally study groups; EG1 (264.05 ± 22.003 pg/ml), EG2 (483.088 ± 18.402 pg/ml), EG3 (744.443 ± 44.525 pg/ml), and EG4 (1046.2 ± 34.712 pg/ml) comparing to those of NCG (151.625 ± 10.32 pg/ml). Also, the highest values of ALT among the experimentally study groups were recorded significantly ($p<0.05$) in EG4 while the lowest was seen in EG1 (Figure 5).



Group	M ± SE
NCG	2.025 ± 0.149
EG1	4.65 ± 0.341
EG2	7.65 ± 0.555
EG3	9.714 ± 0.408
EG4	11.18 ± 0.312
p-value	0.0001 ****
95% *CI*	2.418 to 11.67
R^2	0.9179

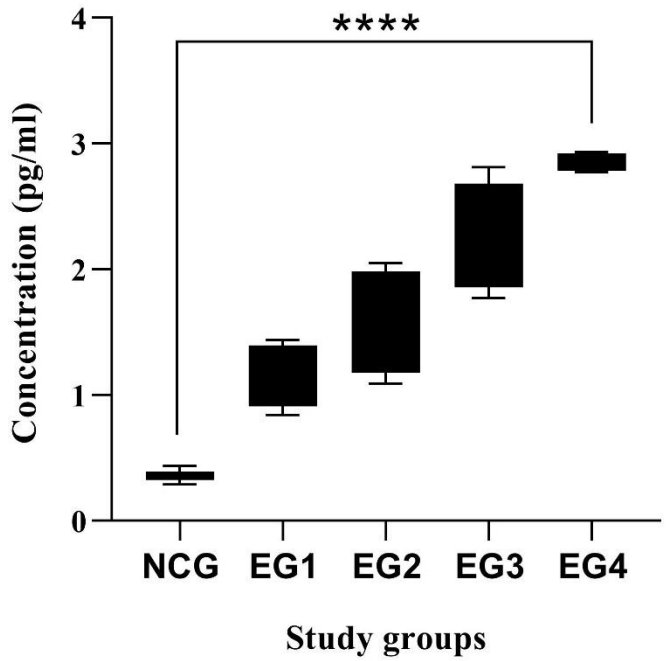
Figure (4): Concentrations of ALP among the rats of five study groups



Group	M ± SE
NCG	151.625 ± 10.32
EG1	264.05 ± 22.003
EG2	483.088 ± 18.402
EG3	744.443 ± 44.525
EG4	1046.2 ± 34.712
p-value	0.0001 ****
95% *CI*	86.66 to 989.1
R^2	0.9542

Figure (5): Concentrations of ALT among the rats of five study groups

In the present study, significant increases ($p<0.0001$) in values of AST were detected in experimentally study groups: EG1 (1.079 ± 0.09 pg/ml), EG2 (1.59 ± 0.138 pg/ml), EG3 (2.217 ± 0.171 pg/ml), and EG4 (2.858 ± 0.036 pg/ml) when compared to those of NCG (0.366 ± 0.018 pg/ml). However, the lowest value of AST was reported significantly ($p<0.05$) in EG1, while the highest was seen in EG4 (Figure 6).



Group	M ± SE
NCG	0.366 ± 0.018
EG1	1.079 ± 0.09
EG2	1.59 ± 0.138
EG3	2.217 ± 0.171
EG4	2.858 ± 0.036
p-value	0.0001 ****
95% *CI*	0.4189 to 2.825
R^2	0.9100

Figure (6): Concentrations of AST among the rats of five study groups

4. Discussion

This work gives insight into the impact of streptozotocin, commonly used to experimentally induce diabetes mellitus, on the levels of antioxidants and liver enzymes. In the current study, the findings revealed that the administration of streptozotocin for rats causes a significant reduction in levels of antioxidants and increases in liver enzymes. Catalase is an antioxidant enzyme that plays a very crucial role against oxidative stress in cells as it decomposes hydrogen peroxide (H_2O_2) into water and oxygen (Gebicka and Krych-Madej, 2019). The effect that administration of streptozotocin has on the catalase activity of different tissues was investigated (Nazaroglu et al., 2009; Chatuphonprasert et al., 2014). Catalase plays a vital role in protecting pancreatic β-cells from damage by free radicals. Pancreatic β-cells are rich in mitochondria, and catalase deficiency in these cells leads to excessive production of ROS, inducing the oxidative stress and cellular dysfunction seen in diabetes mellitus (Wang and Wang, 2017; Eguchi et al., 2021). The mechanism by which streptozotocin induces diabetic effects has been said to be through the production of free radicals, lowering the cellular ATP concentration, and selectively toxic effects on pancreatic β-cells (Eleazu et al., 2013). These processes may also influence the function of GPX that catalyses the reduction of H_2O_2 and organic hydroperoxides and consequently protect cells from oxidative damage (Ighodaro and Akinloye, 2018). Some of the previous and recent experiments have aimed at determining the impact of diabetes evoked by streptozotocin on the alterations of the GPX activity in the liver, kidneys, and testes (Govindaraj and Sorimuthu Pillai, 2015; Othman et al., 2021; Gonçalves et al., 2022).

The overall antioxidant status was observed to be lower in the testes of diabetic rats, and the author also noted that there was a significant decrease in the GPX level. It was also observed that streptozotocin-induced diabetes is caused by increased oxidative stress and DNA damage (Mohasseb et al., 2011). In view of this finding, one may deduce that the ability of antioxidant protective systems in diabetes, particularly decreasing GPX activity, maybe a possible cause for impaired testicular toxicity in diabetic patients. In another study, Haidara et al. (2011) demonstrated the time-course of effects of streptozotocin on hepatic drug metabolism and various liver protein levels concentration of glucose and triglycerides in serum. The researchers stated that while diagnosing diabetes with streptozotocin in 2 hours, the normal glucose level was attained at 8 hours and again augmented at 24 hours. Intriguingly, the increase in serum parameters was not proportional to changes in hepatic parameters, making it possible that streptozotocin has different effects on the liver and serum levels. Akbarzadeh et al. (2018) pointed out that the primary physiological alterations concerning streptozotocin-induced diabetic rats include body weight loss,

decrease in weight of the liver, kidney, heart, and other organs of the body, hyperglycemia, hypoinsulinemia, etc. Streptozotocin administration has a wide-ranging impact on the physiological parameters of the organism.

Earlier literature reviewed pointed to the fact that streptozotocin has an impact on the activity of ALP with regard to the experimental animal models together with the cellular substrate (Rehman et al., 2023). For these reasons, alterations of this enzyme should be discussed as an example of rather universal effects of streptozotocin at the level of various metabolic pathways, including glucose and lipid homeostasis (Dey and Lakshmanan, 2013; Taghipour et al., 2019). Some researchers have used streptozotocin to assess the impact of the method on ALT in types of animals (Voss et al., 1988; Abolfathi et al., 2012; Sarhat et al., 2016). Hattangady and Rajadhyaksha (2009) mentioned that streptozotocin was dependent on the period of treatment and aspects of health indices, including serum glucose and triglyceride levels. Grüßner et al. (1993) reported that diabetes was also induced by treatment with streptozotocin within 2 hours of the treatment, but this was not permanent and hence not observed at 8 hours. Interestingly, streptozotocin was noted to inhibit drug metabolism activity, but its level returned to the baseline 2 hours after the injection and stayed at that level even after 24 hours of injection. Such findings increase the possibility that streptozotocin has the ability to impart a complex and time-of-day-related influence on various metabolic processes, including aspartate aminotransferase reactions.

5. Conclusion

This study revealed the negative effect of streptozotocin on targeted biomarkers, suggesting its role in the development of oxidative stress and related complications in a number of body tissues. Subsequently, the impact is dose-dependent since the high dose of streptozotocin causes severe alteration in the values of all antioxidants and liver enzymes. However, additional studies are of great importance in estimating the effect of streptozotocin on different enzymes, hormones, and body markers, even tissues and organs.

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Conflict of interests: No.

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References

[1] Abolfathi, A. A., Mohajeri, D., Rezaie, A., and Nazeri, M. (2012). Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), 740671.

[2] Akbarzadeh, S., Gholampour, H., Farzadinia, P., Daneshi, A., Ramavandi, B., Moazzeni, A., and Bargahi, A. (2018). Anti-diabetic effects of Sargassum oligocystum on Streptozotocin-induced diabetic rat. *Iranian Journal of Basic Medical Sciences*, *21*(3), 342.

[3] Akhtar, A. (2015). The flaws and human harms of animal experimentation. *Cambridge Quarterly of Healthcare Ethics*, *24*(4), 407-419.

[4] Al-Khatawi, G. M., Mageed, A. H., Albadry, M. A., and Gharban, H. A. (2025). Physiological Impact of Formalin on Lipid Profile, and Protective Role of Vitamin C. *Egyptian Journal of Veterinary Sciences*, *56*(7), 1513-1520.

[5] Aoki, T., Kokudo, N., Komoto, I., Takaori, K., Kimura, W., Sano, K., and Imamura, M. (2015). Streptozocin chemotherapy for advanced/metastatic well-differentiated neuroendocrine tumors: an analysis of a multi-center survey in Japan. *Journal of gastroenterology*, *50*, 769-775.

[6] Arunachalam, K., and Parimelazhagan, T. (2013). Antidiabetic activity of Ficus amplissima Smith. bark extract in streptozotocin induced diabetic rats. *Journal of ethnopharmacology*, *147*(2), 302-310.

[7] Bollard, J., Patte, C., Massoma, P., Goddard, I., Gadot, N., Benslama, N., and Vercherat, C. (2018). Combinatorial treatment with mTOR inhibitors and streptozotocin leads to synergistic In Vitro and In Vivo antitumor effects in insulinoma cells. *Molecular Cancer Therapeutics*, *17*(1), 60-72.

[8] Chatuphonprasert, W., Lao-Ong, T., and Jarukamjorn, K. (2014). Improvement of superoxide dismutase and catalase in streptozotocin–nicotinamide-induced type 2-diabetes in mice by berberine and glibenclamide. *Pharmaceutical biology*, *52*(4), 419-427.

[9] Cummings, F. J., Gelman, R., Skeel, R. T., Kuperminc, M., Israel, L., Colsky, J., and Tormey, D. (1981). Phase II trials of baker's antifol, bleomycin, CCNU, streptozotocin, tilorone, and 5-fluorodeoxyuridine plus arabinosyl cytosine in metastatic breast cancer. *Cancer*, *48*(3), 681-685.

[10] de Vries, R. B., Wever, K. E., Avey, M. T., Stephens, M. L., Sena, E. S., and Leenaars, M. (2014). The usefulness of systematic reviews of animal experiments for the design of preclinical and clinical studies. *ILAR journal*, *55*(3), 427-437.
[11] Dey, A., and Lakshmanan, J. (2013). The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver. *Food and function*, *4*(8), 1148-1184.
[12] Eguchi, N., Vaziri, N. D., Dafoe, D. C., and Ichii, H. (2021). The role of oxidative stress in pancreatic β cell dysfunction in diabetes. *International journal of molecular sciences*, *22*(4), 1509.
[13] Eleazu, C. O., and Eleazu, K. C. (2013). Ameliorating potentials of 3 medicinal plants on relative pancreatic weights in streptozotocin induced diabetic rats. *J Diabetes Metab*, *4*(264), 2.
[14] Ewart, L., Aylott, M., Deurinck, M., Engwall, M., Gallacher, D. J., Geys, H., and Valentin, J. P. (2014). The concordance between nonclinical and phase I clinical cardiovascular assessment from a cross-company data sharing initiative. *Toxicological Sciences*, *142*(2), 427-435.
[15] Faggion Jr, C. M. (2015). Animal research as a basis for clinical trials. *European Journal of Oral Sciences*, *123*(2), 61-64.
[16] Furman, B.L. (2015). Streptozotocin-induced diabetic models in mice and rats. *Current protocols in pharmacology*, *70*(1), 5-47.
[17] Franco, N. H. (2013). Animal experiments in biomedical research: a historical perspective. *Animals*, *3*(1), 238-273.
[18] Garattini, S., and Grignaschi, G. (2017). Animal testing is still the best way to find new treatments for patients. *European journal of internal medicine*, *39*, 32-35.
[19] Gebicka, L., and Krych-Madej, J. (2019). The role of catalases in the prevention/promotion of oxidative stress. *Journal of inorganic biochemistry*, *197*, 110699.
[20] Gharban, H. A. (2024). First genotyping confirmation of Pichia kudriavzevii in subclinically mastitic cows, Iraq: Fungal subclinical mastitis. *Revista de Ciências Agroveterinárias*, *23*(3), 417-424.
[21] Gonçalves, M. G., Medeiros, M. A., de Lemos, L. I. C., de Fátima Campos Pedrosa, L., de Andrade Santos, P. P., Abreu, B. J., and Lima, J. P. M. S. (2022). Effects of creatine supplementation on histopathological and biochemical parameters in the kidney and pancreas of streptozotocin-induced diabetic rats. *Nutrients*, *14*(3), 431.
[22] Govindaraj, J., and Sorimuthu Pillai, S. (2015). Rosmarinic acid modulates the antioxidant status and protects pancreatic tissues from glucolipotoxicity mediated oxidative stress in high-fat diet: streptozotocin-induced diabetic rats. *Molecular and cellular biochemistry*, *404*, 143-159.
[23] Goyal, S. N., Reddy, N. M., Patil, K. R., Nakhate, K. T., Ojha, S., Patil, C. R., and Agrawal, Y. O. (2016). Challenges and issues with streptozotocin-induced diabetes–a clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. *Chemico-biological interactions*, *244*, 49-63.
[24] Grüßner, R., Nakhleh, R., Grüßner, A., Tomadze, G., Diem, P., and Sutherland, D. (1993). Streptozotocin-induced diabetes mellitus in pigs. *Hormone and metabolic research*, *25*(04), 199-203.
[25] Haidara, M. A., Mikhailidis, D. P., Rateb, M. A., Ahmed, Z. A., Yassin, H. Z., Ibrahim, I. M., and Rashed, L. A. (2009). Evaluation of the effect of oxidative stress and vitamin E supplementation on renal function in rats with streptozotocin-induced Type 1 diabetes. *Journal of Diabetes and its Complications*, *23*(2), 130-136.
[26] Hall, E. D., and Traystman, R. J. (2009). Role of animal studies in the design of clinical trials. *Frontiers of Neurology and Neuroscience*, *25*, 10.
[27] Hattangady, N. G., and Rajadhyaksha, M. S. (2009). A brief review of in vitro models of diabetic neuropathy. *International Journal of Diabetes in Developing Countries*, *29*(4), 143.
[28] Hau, J. (2008). Animal models for human diseases: an overview. *Sourcebook of models for biomedical research*, 3-8.
[29] He, H., Liu, L., Morin, E. E., Liu, M., and Schwendeman, A. (2019). Survey of clinical translation of cancer nanomedicines—lessons learned from successes and failures. *Accounts of chemical research*, *52*(9), 2445-2461.
[30] Horton, J., Mittleman, A., Taylor III, S. G., Jurkowitz, L., Bennett, J. M., Ezdinli, E., and Hanley, J. A. (1975). Phase II trials with procarbazine (NSC-77213), streptozotocin (NSC-85998), 6-thioguanine (NSC-752), and CCNU (NSC-79037) in patients with metastatic cancer of the large bowel. *Cancer Chemother Rep*, *59*, 333-340.
[31] Ighodaro, O. M., and Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, *54*(4), 287-293.
[32] La Salvia, A., Bellino, S., and Fanciulli, G. (2024). Streptozotocin in pancreatic neuroendocrine tumors: a focus on efficacy and safety. *Expert Opinion on Pharmacotherapy*, (just-accepted).
[33] Levi, J. A., Wiernik, P. H., and Diggs, C. H. (1977). Combination chemotherapy of advanced previously treated Hodgkin's disease with streptozotocin, CCNU, adriamycin and bleomycin. *Medical and pediatric oncology*, *3*(1), 33-40.
[34] Loewa, A., Feng, J. J., and Hedtrich, S. (2023). Human disease models in drug development. *Nature reviews bioengineering*, *1*(8), 545-559.
[35] Majeed, H. M., Khitam, J. S., Rashad, F. G., and Husham, F. M. (2008). Role of grape seed extract as anti hyperglycemia and antioxidant in experimental diabetic rats. *Journal of Missan Researches*, *4*(8), 1.
[36] Mak, I. W., Evaniew, N., and Ghert, M. (2014). Lost in translation: animal models and clinical trials in cancer treatment. *American journal of translational research*, *6*(2), 114.

[37] McGonigle, P., and Ruggeri, B. (2014). Animal models of human disease: challenges in enabling translation. *Biochemical pharmacology*, *87*(1), 162-171.
[38] Mohasseb, M., Ebied, S., Yehia, M. A., and Hussein, N. (2011). Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. *Journal of physiology and biochemistry*, *67*, 185-194.
[39] Mukherjee, P., Roy, S., Ghosh, D., and Nandi, S. K. (2022). Role of animal models in biomedical research: a review. *Laboratory Animal Research*, *38*(1), 18.
[40] Murray-Lyon, I. M., Cassar, J., Coulson, R., Williams, R., Ganguli, P. C., Edwards, J. C., and Taylor, K. W. (1971). Further studies on streptozotocin therapy for a multiple-hormone-producing islet cell carcinoma. *Gut*, *12*(9), 717-720.
[41] Nazaroglu, N. K., Sepici-Dincel, A., and Altan, N. (2009). The effects of sulfonylurea glyburide on superoxide dismutase, catalase, and glutathione peroxidase activities in the brain tissue of streptozotocin-induced diabetic rat. *Journal of Diabetes and its Complications*, *23*(3), 209-213.
[42] Othman, M. S., Khaled, A. M., Al-Bagawi, A. H., Fareid, M. A., Ghany, R. A., Habotta, O. A., and Moneim, A. E. A. (2021). Hepatorenal protective efficacy of flavonoids from Ocimum basilicum extract in diabetic albino rats: A focus on hypoglycemic, antioxidant, anti-inflammatory and anti-apoptotic activities. *Biomedicine and Pharmacotherapy*, *144*, 112287.
[43] Poduri, R. (2021). Historical perspective of drug discovery and development. *Drug discovery and development: From targets and molecules to medicines*, 1-10.
[44] Rehman, H. U., Ullah, K., Rasool, A., Manzoor, R., Yuan, Y., Tareen, A. M., and Bashir, S. (2023). Comparative impact of streptozotocin on altering normal glucose homeostasis in diabetic rats compared to normoglycemic rats. *Scientific Reports*, *13*(1), 7921.
[45] Sarhat, E. R., Wadi, S. A., and Ibrahim, S. K. (2016, May). The Influence of Lycopene on Interleukin-6, Tumor Necrosis Factor-α, Alanine Aminotransferase, Aspartate Aminotransferase Levels In Stereptozotocin-Induced Diabetic Rabbits. In *3rd Scientific Conference-College of Veterinary Medicine-University of Tikrit* (pp. 1-5).
[46] Shanks, N., Greek, R., and Greek, J. (2009). Are animal models predictive for humans?. *Philosophy, ethics, and humanities in medicine*, *4*, 1-20.
[47] Singh, V. K., and Seed, T. M. (2021). How necessary are animal models for modern drug discovery?. *Expert opinion on drug discovery*, *16*(12), 1391-1397.
[48] Sithole, H. L. (2009). A review of the use of Streptozotocin (STZ) in the induction of diabetes in rats and subsequent ocular tissue changes. *African Vision and Eye Health*, *68*(2), 82-88.
[49] Swearengen, J. R. (2018). Choosing the right animal model for infectious disease research. *Animal models and experimental medicine*, *1*(2), 100-108.
[50] Taghipour, Y. D., Hajialyani, M., Naseri, R., Hesari, M., Mohammadi, P., Stefanucci, A., and Abdollahi, M. (2019). Nanoformulations of natural products for management of metabolic syndrome. *International journal of nanomedicine*, 5303-5321.
[51] Uhl, E. W., and Warner, N. J. (2015). Mouse models as predictors of human responses: evolutionary medicine. *Current pathobiology reports*, *3*, 219-223.
[52] Vashishat, A., Patel, P., Das Gupta, G., and Das Kurmi, B. (2024). Alternatives of animal models for biomedical research: a comprehensive review of modern approaches. *Stem Cell Reviews and Reports*, *20*(4), 881-899.
[53] Voss, C., Brachmann, K., and Hartmann, K. (1988). Effect of Streptozotocin on transaminases, creatinine and urea in serum of rats. *Experimental and Clinical Endocrinology and Diabetes*, *92*(04), 37-42.
[54] Wahab, B. A. A., Merah, M. H., Latif, A. D., and Gharban, H. A. (2024). Alternative therapeutic approach of ovine subclinical mastitis using the ethanolic roots extract of Capparis spinosa. *Open Veterinary Journal*, *14*(3), 814.
[55] Wall, R. J., and Shani, M. (2008). Are animal models as good as we think?. *Theriogenology*, *69*(1), 2-9.
[56] Wang, J., and Wang, H. (2017). Oxidative stress in pancreatic beta cell regeneration. *Oxidative medicine and cellular longevity*, *2017*(1), 1930261.
[57] Zhu, B. T. (2022). Pathogenic mechanism of autoimmune diabetes mellitus in humans: potential role of streptozotocin-induced selective autoimmunity against human islet β-cells. *Cells*, *11*(3), 492.