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Physiological Effects of Alloxan on Serum Glucose Levels and Liver Function Test in Male Rabbit

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ABSTRACT

Diabetes is a metabolic disorder characterized by elevated blood glucose levels that can lead to various complications. Exploring the physiological alterations in rabbits can provide valuable insights for the development of therapeutic interventions. This research delves into the impact of diabetes on the physiological and biochemical parameters of male rabbits. **Objectives:** To compare the physiological parameters like body temperature, heart rate, respiration rate, and oxygen saturation) and body weight and biochemical parameters, including blood glucose levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, lactate dehydrogenase (LDH), and total protein levels in diabetic and non-diabetic rabbits. **Methods:** An experiment on 30 male rabbits divided into diabetic and control groups measured physiological parameters like body temperature, heart rate, respiration rate, and oxygen saturation. Body weight and blood glucose levels were tracked, and blood samples were taken for ALT, AST, creatinine, LDH, cholesterol, triglycerides and total protein levels. Statistical analysis was conducted to compare the physiological and biochemical parameters between the diabetic and control groups. **Results:** The results showed that induced diabetes in male rabbits affects their physiological and biochemical parameters significantly. Diabetic rabbits had lower body temperature, heart rate, respiration rate, and oxygen saturation compared to the control group. They also had higher body weight and blood glucose levels. Biochemical analysis showed increased ALT, AST, and creatinine levels, and decreased LDH and total protein levels in diabetic rabbits. **Conclusions:** These results demonstrate the extensive impact of diabetes on rabbit physiology and biochemistry, offering insights for future diabetes research.

INTRODUCTION

Diabetes is a metabolic disorder characterized by persistent high levels of glucose in the blood due to abnormalities in insulin regulation. It poses a significant global health challenge, leading to mortality, morbidity, and substantial economic consequences. Type I diabetes arises from an insufficient production of insulin, while type II diabetes stems from the body's inability to effectively utilize insulin; both types require treatment for over 40% of affected individuals [1]. It is unsurprising that a substantial amount of research is currently underway, aimed at investigating the etiology, diagnosis, and management of this debilitating condition [2]. Animal testing for diabetes uses toxic chemicals like streptozotocin [3, 4] transgenic rodents used for studying diabetes are becoming increasingly popular. A significant critique of utilizing rodents stems from their abbreviated lifespan, which may preclude the manifestation of clinical complications commonly observed in humans who have suffered from diabetes for a prolonged period. In some cases, felines, canines, porcine species and nonhuman primates are employed as alternatives [5]. Rabbits present a viable and often neglected option for conducting chronic diabetes experiments due to their manageable nature. Rabbits are less phylogenetically advanced than cats, dogs, pigs, and primates. Rabbits live 5-8 years, longer than rodents. As research subjects, rabbits possess advantageous attributes such as their manageable size, extended lifespan, amiable dispositions, ease of handling, and cost-effectiveness [6]. Our extensive exploration of PubMed unveiled that within the past decade, a multitude of

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studies, exceeding 1000 in number, have harnessed rabbits as a viable model for diabetes [7]. Nonetheless, the majority of these investigations involved rabbits with diabetes for a limited duration, typically ranging from weeks to a mere two months [8]. Studies of such brevity have severely restricted the ability to delve into diabetic research, given the multitude of complications linked to diabetes that require a prolonged period to manifest [5]. Elevated blood glucose is a feature of all types of diabetes because of a relative or total lack of the hormone insulin, which is secreted by the islets of Langerhans in the pancreas. Insulin lowers blood sugar and regulates metabolism [9]. Substances influence the production and release of hormone. Nutrient secretagogues raise ATP levels, while non-nutrient secretagogues stimulate brain pathways. Certain sugars do not require insulin to enter cells. Pancreatic secretion is influenced by glucose levels. Insulin binds with a receptor in the cell membrane to activate tyrosine kinase activity [10]. Guides metabolism and muscle transfer, using muscle glycogen for energy and stimulating lipid/glycogen production while inhibiting lipolysis gluconeogenesis. Insulin and growth hormone/IGF-I work together to prevent hypoglycemia, with growth hormone being produced when the extracellular component activates AT. Counter-regulatory hormones such as Glucocorticoids and catecholamines regulate metabolic processes by modulating the activity of enzymes through phosphorylation/dephosphorylation mechanisms, which are influenced by the balance between insulin and glucagon levels [11]. We have established a robust rabbit model for type 1 diabetes research. Alloxan disrupts pancreatic cells, resulting in hyperglycemia. This model proves to be an effective tool in exploring new treatments for diabetes.

This study aimed to investigate the effects of this disease on rabbits, and this paper expounds upon management strategies, as well as the physiological, biochemical, and hepatic glucose test results of the rabbits.

METHODS

A total of 30 young male rabbits of 8–10 weeks old with an average weight of 1.85 ±0.13 kg was divided into two groups: 15 with diabetes and 15 healthy. They were housed individually in cages with controlled temperature and humidity, and provided with a specific diet and water. Environmental enrichment was also provided. The animals were parasite-free and acclimatized for at least seven days before experimentation began. Rabbits were systematically weighed on a weekly basis over the six-week duration of the study, with their weights being meticulously documented. Prior to the administration of alloxan, the rabbits were gently anesthetized with ketamine hydrochloride at a dosage of 30 mg/kg and xylazine at 3 mg/kg (administered intramuscularly). To prevent dryness, a carefully applied ointment of artificial tears (Butler Animal Health Supply, Dublin, OH) was administered to the surface of each eye. The rabbit cohorts were induced into a diabetic state through the administration of a solitary intravenous dose of 150 mg/kg of alloxan monohydrate (Sigma, St. Louis, MO) dissolved in 0.9% NaCl [12]. The rabbit's body temperature was meticulously maintained using a warm water circulating blanket (Gaymar T Pump, Gaymar Industries Inc., Orchard Park, NY). Parameters such as heart rate, respiratory rate, body temperature, and SpO2 were closely monitored while the animals were under anesthesia and during their recovery. Alloxan monohydrate was dissolved in saline and administered to rabbits intravenously. To prevent hypoglycemia, glucose was administered at intervals and oral glucose solution provided. Rabbits with low blood glucose levels received a second dose of alloxan to maintain levels above 300 mg/dl. Rabbits injected with alloxan to induce diabetes had blood glucose monitored regularly. Insulin given based on glucose levels. Samples collected weekly for one months of experiment. Insulin dosage for rabbits determined by blood glucose curve after trial dose. Final dosage set if peak BGL over 350 mg/dl and trough BGL 50 mg/dl or higher. Illness symptoms prompt new curve. Regular monitoring with blood glucose meter. Morning BGL over 350 mg/dl, Novolin-R insulin administered SC daily. Blood specimens were obtained for examination. The blood was drawn from the central auricular artery using a 25-gauge needle. Plasma was acquired through the process of centrifugation of the blood at 2500 g for 20 minutes at a temperature of 4°C, and was subsequently stored at -20°C. The aforementioned analyzer was employed to assess levels of LDH, AST, ALT, total protein, cholesterol, TG, BUN, and creatinine. The examination was performed using the Roche Cobas Mira Plus chemistry analyzer and associated reagents. The results were presented as the mean value accompanied by its corresponding standard error (SEM). The Student's t-test was used to compare the two groups using statistical software from GraphPad Prism Software, Inc. based in San Diego, California. A significance threshold of $P < 0.05$ was deemed statistically significant.

RESULTS

Body weight of Group A (induced) and Group B (Control) rabbits were observed before and after 6 weeks of induction. Significant difference ($P<0.05$) was observed in body weight of rabbits among A and B groups (Figure 1).

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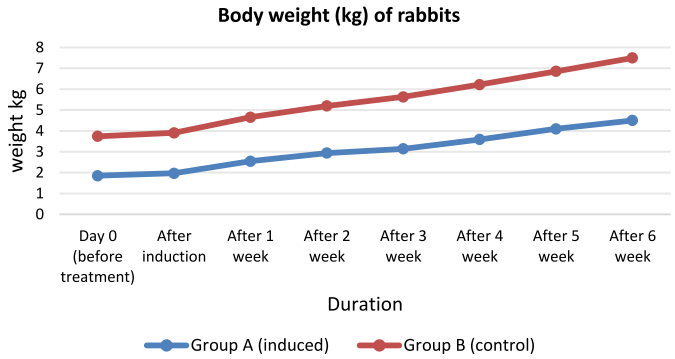

Figure 1: Body weight (kg) of Rabbits from Day 0 to 6-week Duration in Group A(Induced)and Group B(Control).

Different physiological parameters such as body temperature, heart rate, respiration rate and oxygen saturation of Group A (induced) and Group B (Control) rabbits were observed. There was a decline in body temperature, heart rate, respiration rate and oxygen saturation of induced rabbits as compared to non-induced rabbits. Significant difference (P<0.05) was observed in body temperature, heart rate, respiration rate and oxygen saturation of rabbits among A and B groups(Figure 2).

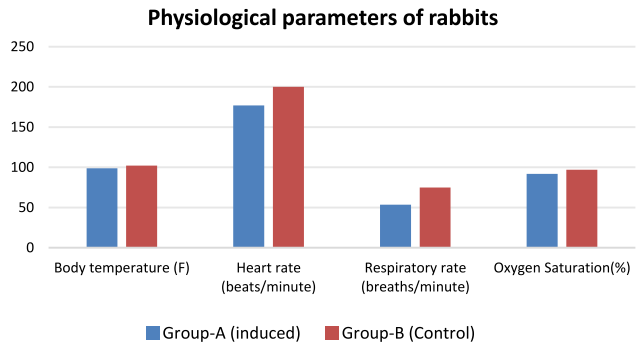

Figure 2: Physiological Parameters of Rabbits

Blood glucose level of Group A (induced) and Group B (Control) rabbits were observed before and after 24 hours of treatment. Significant difference (P<0.05) was observed in blood glucose level of rabbits among A and B groups (Table 1).

Table 1: Hourly Blood Glucose Level of Rabbits

Host	Group A (induced) Mean ± SEM	Group B (control) Mean ± SEM
Day 0 (before treatment)	141.4 ± 6.44[a]	144.3 ± 5.82[a]
After 2 hours of Alloxan Injection	350.20 ± 18.99[a]	142.4 ± 4.33[b]
After 4 hours of Alloxan Injection	280.64 ± 15.46[a]	141.3 ± 4.22[b]
After 8 hours of Alloxan Injection	211.55 ± 11.36[a]	145.1 ± 3.85[b]
After 12 hours of Alloxan Injection	200.84 ± 8.33[a]	138.2 ± 2.33[b]
After 24 hours of Alloxan Injection	196.35 ± 5.33[a]	140.3 ± 5.66[b]

Alphabets (a, b) among the mean indicates significant (P<0.05) difference between the groups.

Blood glucose level of Group A (induced) and Group B (Control)rabbits were observed before and after 4 weeks of induction. Significant difference (P<0.05) was observed in blood glucose level of rabbits among A and B groups (Table 2).

Table 2: Weekly Blood Glucose Level of rabbits

Duration	Group A (induced) Mean ± SEM	Group B (control) Mean ± SEM
Week 1	205.44 ± 15.4[a]	150.5 ± 3.58[b]
Week 2	200.66 ± 11.52[a]	147.4 ± 2.33[b]
Week 3	198.97 ± 6.39[a]	145.3 ± 2.52[b]
Week 4	199.5 ± 5.22[a]	142.1 ± 2.11[b]
Week 5	201 ± 4.2[a]	140 ± 2.22[b]
Week 6	205 ± 3.2	141 ± 3.2[b]

Alphabets (a, b) among the mean indicates significant (P<0.05) difference between the groups.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) of Group A (induced) and Group B (Control) rabbits were determined before and after injecting Alloxan. ALT and AST values of induced rabbits were increased as compared to control group rabbits. Significant difference (P<0.05) was observed in ALT and AST of rabbits among A and B groups(Table 3).

Table 3: Liver Function Test of Rabbits

Duration	Group A (induced) Mean ± SEM	Group B (control) Mean ± SEM
ALT (U/L) Before	21 ± 1.55[a]	20.6 ± 1.43[a]
ALT (U/L) After Alloxan Injection	37.8 ± 2.66[a]	20.6 ± 1.50[b]
AST (U/L) Before	97 ± 1.59[a]	97 ± 1.66[a]
AST (U/L) After Alloxan Injection	195 ± 2.55[a]	97 ± 1.64[b]

Alphabets (a, b) among the mean indicates significant (p<0.05) difference between the groups.

Plasma lactate dehydrogenase (LDH), creatinine and total protein levels of Group A (induced) and Group B (Control) rabbits were determined before and after injecting Alloxan. The results showed reduction in LDH and total protein levels of induced rabbits as compared to non-induced rabbits. Significant difference (P<0.05) was observed in LDH and total protein of rabbits among A and B groups. Creatinine of induced rabbits was increased as compared to control group rabbits. Significant difference (P<0.05) was observed in creatinine of rabbits among A and B groups (Table 4).

Table 4: Serum Biochemical Parameters in Diabetic and Control Group Rabbits

Duration	Group A (induced) Mean ± SEM	Group B (control) Mean ± SEM
LDH (mg/dl) before	69.44 ± 1.14[a]	69.55 ± 1.23[a]
LDH (mg/dl) after Alloxan Inj.	123 ± 2.33[b]	70.11 ± 4.66[a]
Creatinine (mg/dl) value before	1.264 ± 0.13[a]	1.257 ± 0.12[a]
Creatinine (mg/dl) after Alloxan Inj.	2.476 ± 0.88[a]	1.261 ± 0.09[b]
Total protein(mg/dl) before treatment	5.50 ± 1.44[a]	5.54 ± 1.22[a]
Total protein (mg/dl) after Alloxan Inj.	7.80 ± 1.53[a]	5.40 ± 1.64[b]

The blood urea, triglyceride and cholesterol levels of

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rabbits in the Group A (induced) and Group B (Control) were assessed both before and after the administration of Alloxan. Th results showed a decrease in urea, triglyceride and cholesterol levels in the induced rabbits as compared to the non-induced rabbits. A statistically significant difference ($P<0.05$) was noted in the urea, triglyceride and cholesterol levels between groups A and B (Figure 3).

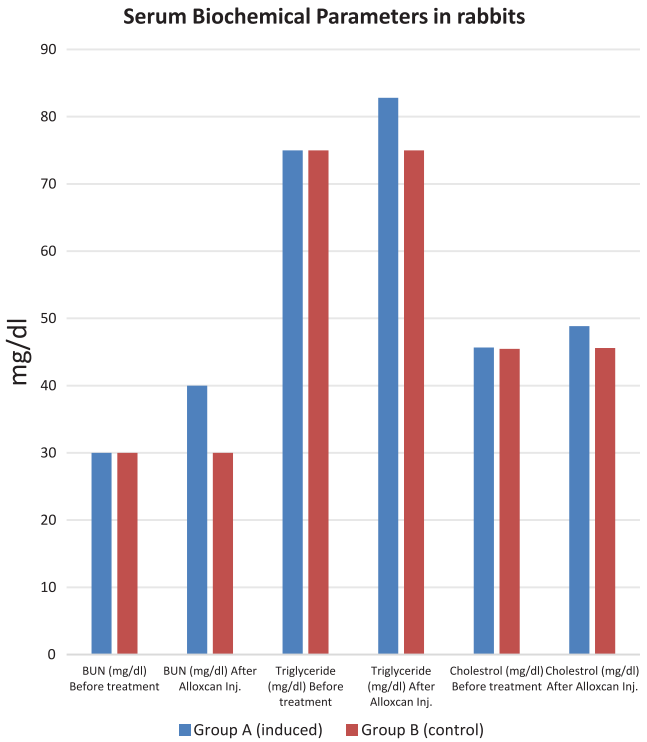

Figure 3: Comparison of Blood Urea, Triglyceride and Cholesterol Levels in Induced and Control Group Rabbits

DISCUSSION

In recent years, there has been a surge in research delving into the impacts of diabetes on rabbits during relatively short timeframes. However, the utility of these investigations is inherently constrained by the use of short-term diabetic rabbit models, given that human diabetes is characterized by its chronic nature and the gradual onset of diabetic complications. A perusal of the PubMed database yielded a limited number of studies in which diabetic rabbits were maintained for 3-6 months for the purpose of pharmacological or growth factor research [13], with only a singular study documenting the year-long survival of three alloxan-induced diabetic rabbits for the evaluation of their aortic intima-media [14]. Arif *et al.* [15], studied the effects of alloxan on kidney injury in toxin-induced diabetic rabbits. The treated animals showed unconsciousness, hypothermia, high blood urea, and low blood glucose levels. Hyperglycemia and pancreatic islet necrosis were observed, leading to the rapid induction of diabetes in animals like rats. This research indicates similarities between animal and human diabetes symptoms [16, 17]. The onset of diabetes was correlated with a significant elevation in plasma glucose concentrations. The high blood sugar levels observed in Tables 1 and 2 are linked to a notable decrease in insulin levels in fully developed diabetes mellitus as well as a decrease in glucose uptake by muscle and fat cells [18]. The higher glucose levels in the diabetic group supplemented with starch clearly stem from dietary factors. Research has indicated that total carbohydrate intake is a reliable predictor of postprandial glucose levels [19, 20]. The glucose concentration exhibited a notable increase in diabetic rabbits (as illustrated in table 1 & 2). The glucose concentration exhibited a notable increase in diabetic rabbits (as illustrated in table 3 & 4). Flockhart & Larsen [21] illustrated variations in blood sugar levels and insulin sensitivity among the elderly, showing elevated levels, while Bando *et al.* [22] found no seasonal fluctuations in glucose levels. The heightened glucose concentration observed in diabetic rabbits. Furthermore, a decline in physical activity during the winter months has been documented in various studies [23]. This situation necessitates a rapid release of insulin from deteriorating beta cells, ultimately resulting in a hypoglycemic state around six hours after injection, particularly in animals that have been fasting. This increased hypoglycemia is highlighted [24]. After the administration of Alloxan into an animal's body [4] explains that blood glucose levels follow a predictable pattern, increasing within the first two hours primarily due to the breakdown of liver glycogen [25]. The response to toxic and diabetogenic doses varies greatly among different species and even within the same species when it comes to the use of Alloxan [26]. Therefore, the safe diabetogenic dosage is relatively restricted in each animal, as even a slight overdose can be harmful, eventually leading to death primarily due to renal tubular cell necrosis resulting from high doses administered. In this study, a less toxic/diabetogenic dose of 100mg/kg was used, but it did not always result in persistent diabetes in the tested rabbits, as sixty percent required a secondary dose to maintain chronic hyperglycemia, although all rabbits survived until the end of the experiment. Additionally, recovery from this condition could occur either through the multiplication of surviving beta cells after the initial injection or through the production of new beta cells generated from the exocrine pancreas' duct epithelium [27]. The slight increase in serum total protein levels in diabetic rabbits (Tables 4) is generally in line with previous studies [28, 29] that have shown elevated serum total protein levels in diabetic rats. Conversely, other researchers [28, 30] have reported a notable decrease in total protein levels in alloxan-induced diabetic rats. However, our findings indicate that diabetic rabbit groups exhibited lower levels of albumin. Hypoalbuminemia is a

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prevalent issue in diabetic animals and is often associated with diabetic nephropathy [30]. Microalbuminuria is frequently used as a diagnostic marker for early-stage diabetic nephropathy in humans [31]. The decline in serum total protein levels in diabetics has been linked to the inhibition of oxidative phosphorylation, leading to reduced protein synthesis, increased catabolic processes, and diminished protein absorption [31]. The decrease in serum total protein levels in both diabetic and non-diabetic groups (Table 4 & Fig. 3). Previous research has documented a decrease in serum total protein levels in rabbits exposed to heat stress [32, 33]. In the diabetic rabbit group, elevated serum urea levels were sustained (Figure 3). This discovery is in line with studies that have documented increased urea levels in alloxan-induced diabetic rabbits [34, 35] and diabetic individuals [36, 37]. The surge in urea nitrogen in diabetes can be attributed to heightened catabolism of both hepatic and plasma proteins that coincide with gluconeogenesis [38]. It has been proposed that the increase in urea synthesis in streptozotocin-induced diabetes in rats is a result of enzyme induction by glucagon [39]. The higher serum urea level in starch-supplemented diabetic rabbits in the current investigation is congruent with other studies [40] that have reported heightened urea levels in rabbits fed a high carbohydrate-low fat diet. The escalation of urea observed during winter in the present study may be linked to increased food consumption by rabbits and/or hemoconcentration. Elevated serum urea levels were also observed in New Zealand White rabbits [41]. The data suggests that diabetic rabbits exhibited decreased levels of serum creatinine (Table 4). This discovery aligns with previous studies [28, 29] that demonstrated a significant reduction in creatinine levels in alloxan-induced diabetic rats. Analysis of the impact of a high carbohydrate-low fat diet in rabbits showed a marked decrease in serum creatinine levels [41]. However, elevated levels of creatinine may be attributed to conditions related to extensive muscle breakdown, as observed in poorly managed diabetes mellitus [42]. The heightened creatinine levels in the test groups (Table 3) could be linked to increased tissue breakdown associated with elevated levels of glucocorticoid hormones. The current response of creatinine in rabbits corroborates earlier research [43]. Furthermore, diabetic rabbits exhibited elevated serum cholesterol levels (Figure 3). The escalation in cholesterol levels, linked to insulin deficiency, is attributed to heightened plasma concentrations of VLDL and LDL. Similarly, previous studies have documented a rise in cholesterol levels in experimentally induced diabetes in alloxan-diabetic rats [44].

CONCLUSIONS

Based on the findings delineated in this research, it is deduced that the induction of diabetes in rabbits through alloxan administration results in noteworthy modifications in blood biochemical, physiological parameters, and liver function evaluations. Biochemical analysis showed increased ALT, AST, and creatinine levels, and decreased LDH and total protein levels in diabetic rabbits. Diabetic rabbits had lower body temperature, heart rate, respiration rate, and oxygen saturation compared to the control group. They also had higher body weight and blood glucose levels. Additional investigations are necessary to elucidate the concomitant variations in electrolyte levels and acid-base equilibrium.

Author's Contribution

Conceptualization: IAP, ABK
Methodology: IAP
Formal analysis: RSB
Writing, review and editing: JS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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