Khalij Libya Journal of Dental and Medical Research. 2025;9(1):38-43
https://doi.org/10.47705/kjdmr.25911006
eISSN:2708-888X

Original article

Consequences of Alloxan-Induced Diabetes on certain Hematological and Hepatic parameters in Albino Mice

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Abstract
Alloxan is widely used to induce diabetes in animal models because it selectively destroys pancreatic beta islets, which correlate with inverse change in the plasma concentration of insulin, resulting in beta cells necrosis. Diabetes mellitus is a metabolic disorder which affects humans of all ages and has become epidemic in the last few years. Oxidative stress in DM can contribute to various abnormalities in the physiology of blood cells and liver impairment. The primary goal of this study is to investigate how diabetes affects blood parameters and liver function in an animal model, which contributes to managing various complications in diabetic patients. Twenty male Swiss albino mice were housed at research lab in pharmacology department, faculty of medicine, Benghazi. Mice were fasted for 48 hours and then classified into two groups; the first group did not receive any treatment while the second group received a single intraperitoneal injection of freshly prepared alloxan 200mg/kg dissolved in normal saline (PH 4.5) with a concentration of 1%. After one month, the blood samples were collected from all mice and analyzed. The main outcome is that induction of diabetes in albino mice result in elevated blood glucose level, weight loss, low RBC and WBC counts, elevated platelets count, and liver impairment. The findings of this study align with previous research investigating that diabetes can result in anemia, immune suppression, and liver damage. However, translational explanations must account for species-specific responses and model limitations.
Keywords*: Mice, Alloxan, Diabetes, Hematological Parameters, Liver Enzymes.*

Received*: 25/12/24*
Accepted*: 13/02/25*
Published*: 23/02/25*

Introduction

Alloxan is a urea derivative, selectively destroys the insulin-producing β-cells in pancreatic islets, causing their necrosis. This method has been extensively employed to induce experimental diabetes in various animal species, including rabbits, rats, mice, and dogs. The severity of the induced diabetes can be modulated by adjusting the alloxan dose administered [1,2].

The alloxan-induced diabetes model is used to investigate various aspects of diabetes, including its progression, complications, and potential therapeutic interventions. This approach provides valuable insights into the disease, contributing to the development of new treatments and management strategies for Type I diabetes mellitus. The mechanism of alloxan as a toxicant used to induce hyperglycemia in experimental animals involves its toxic effect on the beta cells of the pancreatic islet. Consequently, Reactive oxygen species (ROS) are formed during this process and a cascade of reactions occurs leading to increased levels of superoxide radicals, hydrogen peroxide, and hydroxyl radicals with potential damaging effects on cell macromolecules in the animals [3].

Over the past few years, the prevalence of diabetes mellitus (DM), a metabolic infirmity which becomes a critical health situation, has dramatically elevated over the world [4]. Regarding the International Diabetes Federation record, 2.8% of individuals have diabetes, and by 2030, the percentage is predicted to reach 4.4% [5]. Cellular, hematological, as well as metabolic disorders are linked to uncontrolled diabetes mellitus and can result in vascular complications [6]. The coagulation systems and all the blood cells including red blood cells (RBCs), white blood cells (WBCs) as well as platelets (PLTs) undergo alterations in their structure, metabolism as well as function in diabetic individuals [7]. A decline in the RBC numbers, hemoglobin (Hgb), as well as hematocrit (HCT) levels relative to healthy people may be an indication of these alterations as well as immunological and coagulation issues [8]. The most common hematological alteration in diabetic patients is anemia which is not always noticed [9-11].

The formation of advanced glycation end products (AGEs) and the enhanced generation of reactive oxygen species in long term hyperglycemia are considered as the most common causes of hematological alterations in diabetic patients. Oxidative stress, which is associated with tissue damage and hematological alterations including RBC malfunction, hyperactivity of platelets, dysfunction of endothelial cells, is caused by

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enhanced generation of ROS [12,13]. In diabetic patients, these alterations can result in various problems including hypercoagulability, anemia as well as cardiovascular complications [14]. Insulin resistance as well is associated with malfunction of endothelial cells, exaggerated levels of inflammatory markers as well as platelets hyperactivity, and can speed up vascular problems in diabetic patients [13].
Epidemiological research provides a correlation between the count of WBCs and the risks associated with diabetes, and an enhanced count of WBCs is an indicator of inflammation [15]. Proper homeostasis is maintained by platelets, and MPV is an indicator of platelets' function [16]. In diabetic patients, the atherothrombosis procedure results in development of CVD, platelet hyperactivity is crucial in inflammation, and developed atherosclerosis makes the illness worse [17]. Diabetic individuals with retinopathy, nephropathy as well as coronary cardiac disease experience an elevated level of MPV, which is an indicator of alterations in either platelet activation or the amount of platelet generation [18,19].
Attention has long centered on the liver in diabetes mellitus because of the importance of this organ in carbohydrate metabolism and regulation of blood sugar. Two studies revealed the occurrence of hepatic changes in certain situations of diabetic individuals [20, 21]. Research indicates a significant association between type 2 diabetes mellitus (T2DM) and elevated liver enzymes. Diabetic patients have consistently shown higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) in comparison with healthy individuals [22, 23,24]. These elevated enzymes are crucial markers of hepatic cells injury and may indicate underlying non-alcoholic fatty liver disease (NAFLD) in T2DM patients [23]. Overweight and insulin resistance further exacerbate liver enzyme abnormalities in T2DM [25]. ALT and GGT show significant positive correlations with glycemic control parameters and lipid profiles, suggesting their potential as screening tools for liver abnormalities in T2DM [24]. Regular monitoring of liver enzymes in those patients is recommended for early detection of liver dysfunction and to prevent disease progression [23, 24]. This research was aimed to study the changes in hematological and hepatic parameters in diabetic mice using alloxan to induce diabetes.

Materials and Methods

Materials and animals

Alloxan monohydrate (ALFA AESAR), is obtained from the department of pharmacology, Benghazi university. Diethyl ether 99.8% (Sigma-Aldrich, Germany). Twenty Male Swiss albino mice (SWR) weighing 20-35gm, bred at Research Lab in Department of Pharmacology-Faculty of medicine-University of Benghazi, Benghazi, Libya. Animals were housed in groups to acclimatize to the laboratory conditions before the start of the experiment, as for room temperature, relative humidity, and light cycle. The animals were fed on food pellets and had free access to water.
Diabetes mellitus was induced by single intraperitoneal (IP) injection of freshly prepared 200mg/kg of Alloxan monohydrate dissolved in normal saline (PH 4.5) with a concentration of 1**%** After 48 hrs., the blood samples of mice were gathered from the end part of their tails to measure blood glucose level.

Experimental design

Mice were separated into 2 groups of n = 10 each, the treatment procedure, their dosing, and route of administration are given in Table 1.

Table 1. Design of Experiment

Group No.	Group	No. of Mice	Treatment
I	Control (C)= Healthy untreated mice (Non-diabetic Mice)	10	No treatment. Normal food and water for 30 days.
II	Diabetic Mice Alloxan - induced diabetes (AX)	10	Fasted Mice received a single dose of alloxan (200 mg/kg, IP injection (after 2 days, if blood glucose level exceeds 200mg/dl, it means diabetic mice)

After 30 days of alloxan treatment, the animals were anesthetized under light diethyl ether anesthetic agent. The blood was collected directly from the retro-orbital venous plexus, then the samples of blood were putted in two types of tubes; The first is heparinized tube for measuring complete blood counts (CBCs) and the second tubes for measuring liver function tests, the blood immediately centrifuged at 4.5~10rp~m and Serum was isolated and kept at -20°C until used for biochemical assessment.
The body weight of the control and treated mice were measured at initiation and different time intervals of the experiment. An electronic glucometer (Accua-check®) plus test strips was utilized to calculate blood

Khalij Libya Journal of Dental and Medical Research. 2025;9(1):38-43
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eISSN:2708-888X

glucose concentration. Blood is applied to the end tip of the test strip and then inserted into the glucometer to measure the glucose concentration automatically.
Animals with blood glucose levels more than 200 mg/dl were considered diabetic and used for further experiments [26]. Blood samples were collected from mice into heparinized tubes. An aliquot of blood samples was subjected to complete blood counts which include important hematological parameters by using UniCel DxH 800 (Beckman Coulter, USA) at Al-AKEED Lab, Benghazi. White blood cells (WBCs) number, red blood cells (RBCs) number, hematocrit (HCT), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC), were measured on Hematology Analyzer.
According to Reitmann and Frankel method (1957), The liver enzymes (AST, ALT and ALP) were determined spectrophotochemically, and Malloy and Evelyn method (1937) was used for determination of total bilirubin in serum [21].

Statistical analysis
The results are expressed as mean ± SEM. The statistical evaluation of all data was done using analysis of variance (ANOVA). *p*-value < 0.05 was considered statistically significant. Data was analyzed by SPSS software, version 26.

Results
The diabetic group exhibited a dramatic decline in body weight after 4weeks, which valued 21.833 ± 2.05616g compared to the findings in the non-diabetic mice (29.83 ± 1.447g) as shown in table 2.

Table 2. Body weight of mice in diverse groups

Groups	Group	Mean± SEM
NO treatment	C	29.83±1.447
Animals treated with alloxan only (diabetes induced animals)	AX	21.83±2.056*

In addition, the present results showed significant increases (P < 0.05) in the mean values of blood sugar level of the diabetic group which reached (436.67±37.554mg/dl) 48hours post-treatment with 200mg/kg of Alloxan IP injection, while in untreated control group was (130±7.742mg/dl) as shown in table 3.

Table 3. Effect of alloxan and gum Arabic on FBG (mg/dl) in mice.

Groups	Group	Mean± SEM
NO treatment	C	130.00±7.742
Animals treated with alloxan only (diabetes induced animals)	AX	436.67±37.554*

**Sig. AX compared with C (P < 0.05)*

In this survey, the blood components which measured were the No and percentage of lymphocytes, the No and percentage of granulocytes (Table 4), RBC counts, Hemoglobin, Hematocrit, MCV, MCH and MCHC (Table 5) and platelet counts and MPV (Table 6). The findings of this survey confirmed that the blood components in the alloxan treated group were not identical to those in the untreated group.

Table 4. The number and percentage of Lymphocytes and Granulocytes.

Groups	Total No of WBCs	Lymphocytes		granulocytes	
		No	%	No	%
C	10.55±3.005	8.95±0.902	84.90±1.476	.48±0.0660	4.51±0.366
AX	7.00±6.515	5.67±2.6109	74.50±7.548	0.575±0.210	9.68±3.775

Table5. Effect alloxan on total number of RBC, Hemoglobin and Hematocrit, MCV, MCH and MCHC.

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eISSN:2708-888X

Groups	RBC counts10^6/ µl)	Hemoglobin(g/ dl)	Hematocrit (%)	MCV (fl)	MCH(Pg)	MCHC (%)
C	7.399±0.947	13.113±0.161	48.38±0.677	55.86±0.695	15.15±0.321	27.13±0.394
AX	2.42±1.634	12.95±0.696	50.40±3.039	55.12±1.370	14.18±0.43	25.77±0.297

Table 6. Platelets parameters.

Groups	No (10^3 /ml	MPV (fl)
C	733.50±53.938	6.46±0.108
AX	823.00±116.226	6.22±0.247

Our results showed that, the enzymatic activity of ALT and AST were high at $p<0.05$ when compared to the control (313.670 ± 125.850 IU/L and 121.67 ±18.780, respectively), the creatinine level was high as well (0.067±0.033) as illustrated in table 7.

Table 7. Effect of alloxan on enzymatic activities and total bilirubin levels.

Groups		**ALT(IU/L).**	**AST (IU/L).**	**ALP(IU/L).**	**Total Bilirubin (mg/dl)**
NO treatment	C	93.25±3.198	164.25±9.760	132.25±6.421	0.2±0.408
Animals treated with alloxan only (diabetes induced animals)	AX	121.67±18.780*	313.67±125.850*	176.67±21.419	0.067±0.033*

**Sig. AX compared with C (P < 0.05)*

Discussion

The primary goal of this survey was to explore the effects of alloxan- induced diabetes on hematological and hepatic parameters in albino mice, indicating significant changes that reflect abnormalities seen in diabetic patients. The findings provide a window into systemic dysregulation caused by elevated glucose level and oxidative stress, helping to understand diabetes-related pathophysiology.

In diabetic group, blood Sugar level (436.67 ± 37.55mg/dl vs. 130.00 ± 7.74mg/dl in controls, $p < 0.05$) and weight loss (21.83 ± 2.06 gm in diabetic mice vs 29.83 ± 1.45 gm in controls) were significantly higher. These outcomes confirm the successful trigger of diabetes via alloxan which selectively destroys pancreatic B-cells, impairing insulin secretion and glucose homeostasis [4]. Weight loss aligns with human diabetes, where catabolic states from insulin deficiency can result in muscle atrophy and lipolysis [27].

In addition, diabetic mice showed a reduced RBC count (2.42 x 10^6 /µl vs. 7.40 x 10^6/µl), as well as hemoglobin (12.95 g/dl vs. 13.11g/dl), and these changes correlate with oxidative tension and advanced glycation end products (AGEs), which impair erythropoiesis and RBC survival [7, 8].

In contrast to human data on diabetes and increased WBC numbers [15], diabetic mice were leukopenia (7.00 x10^3/ ml vs. 10.55x10^3/ml). This variation could be due to alloxan's myelosuppressive properties or acute phase stress [1].

Our results showed an increase in platelet count (823 x 10^3/ml vs. 733x 10^3/ml), apart from a decrease in mean platelet volume (MPV) 6.22 fl vs. 6.46 fl). Increased platelets count correlates to the increased risk of hypercoagulability in diabetes [13], but reduced MPV contrasts with human data where higher MPV predicts cardiovascular complications [18]. This may reflect compensatory platelet production or species-species responses. Furthermore, diabetic mice expose elevated liver enzymes (GPT: 121.67 IU/ L vs. 164.25 IU/L) which reflects the damage in hepatocytes. Chronic hyperglycemia raises hepatic oxidative stress and mitochondrial malfunction [20]. The dramatic rise in GOT suggests potential cardiac or skeletal muscle involvement, as GOT is not liver specific [4].

Conclusion

The primary goal of this survey is to explore how diabetes influences blood components and hepatic enzymes in animal models with diabetes, which has many implications for managing diabetes in humans. Although the outcomes provide crucial contributions to understanding oxidative stress and systemic dysregulation, explanatory frameworks on translation must consider responses to varied species. Incorporating hematological, biochemical, and histological examination will boost the clinical relevance of diabetes research during the pre-symptomatic phase.

https://doi.org/10.47705/kjdmr.25911006
eISSN:2708-888X

Conflict of interest

There are no financial, personal, or professional conflicts of interest to declare.

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المستخلص

يستخدم الألوكسان على نطاق واسع للحث على مرض السكري في النماذج الحيوانية لأنه يدمر بشكل انتقائي جزر بيتا البنكرياسية، التي ترتبط بالتغير العكسي في تركيز الأنسولين في البلازما، مما يؤدي إلى نخر خلايا بيتا. داء السكري هو اضطراب أيضي يصيب البشر من جميع الأعمار، وقد أصبح وباءً في السنوات القليلة الماضية. يمكن أن يساهم الإجهاد التأكسدي في حدوث تشوهات مختلفة في فسيولوجيا خلايا الدم وضعف الكبد. الهدف الأساسي من هذه الدراسة هو دراسة كيفية تأثير مرض السكري على بارامترات الدم ووظائف الكبد في نموذج حيواني، مما يساهم في إدارة المضاعفات المختلفة لدى مرضى السكري. تم إيواء عشرين فأرا سويسريا ذكرا في مختبر الأبحاث بقسم الصيدلة بكلية الطب بنغازي. تم صيام الفئران لمدة 48 ساعة ثم تم تصنيفها إلى مجموعتين؛ لم تتلق المجموعة الأولى أي علاج بينما تلقت المجموعة الثانية حقنة واحدة داخل الصفاق من الألوكسان الطازج 200 ملغم / كغم مذاب في محلول ملحي عادي وبعد شهر واحد، تم جمع عينات الدم من جميع الفئران وتحليلها. والنتيجة الرئيسية هي أن تحفيز مرض السكري في الفئران البيضاء يؤدي إلى ارتفاع مستوى السكر في الدم، وفقدان الوزن، وانخفاض عدد كرات الدم الحمراء وخلايا الدم البيضاء، وارتفاع عدد الصفائح الدموية، وضعف الكبد. تتوافق نتائج هذه الدراسة مع الأبحاث السابقة التي بحثت في أن مرض السكري يمكن أن يؤدي إلى فقر الدم وتثبيط المناعة وتلف الكبد. ومع ذلك، يجب أن تأخذ التفسيرات الترجمية في الاعتبار الاستجابات الخاصة بالأنواع والقيود النموذجية.