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SHORT COMMUNICATION article

# Alloxan blood levels and the risk of diabetes mellitus in children

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***Keywords:*** Alloxan, diabetes mellitus, Libya

**Abstract:** Diabetes mellitus is the most common health disorder and one of the leading causes of death. It is such a sort of disorder in which the patients are at all the time on risk of complications. Numerous investigations discovered that oxidative stress plays an important role in the development of vascular complications in diabetes particularly type 2. There are several chemically induced animal models of type 1 diabetes mellitus. The most common chemicals used to generate type 1 diabetes mellitus animals are alloxan and streptozotocin. Alloxan is added to food materials, especially to the all-purpose flour (maida) to bring softness and white color to the flour. Hence, consuming foodstuffs made from this flour can lead to diabetes mellitus. Measuring the concentration of alloxan in the blood of children with type 1 diabetes. 45 volunteers (children 5-15years) in this study. 15 healthy and 30 patients with diabetes mellitus (females: 53.0%, and males: 47.0%). Venous blood was taken from the elbow vein and transferred as soon as possible into deproteinization solution without any contact with oxygen. Average blood levels of alloxan are determined by the Archibald spectrophotometric method. The mean level of alloxan between healthy and diabetic groups statistically is insignificant (Median=0.458) and diabetic group (Median=0.806). Low-carbohydrate diet low adherence diabetics (46.6%, median=1.91), low- carbohydrate diet moderate adherence diabetics (26.6%, median=0.89), low-carbohydrate diet high adherence diabetics (26.6%, median=0.1). The findings indicate that the alloxan level is higher in healthy people compared to diabetic volunteers whose follow a low-carbohydrate diet, in addition to the reducing of their pastry intake.

## Introduction

Diabetes is the most common health disorder around the world and one of the leading causes of death [1]. The World Health Organization indicates that the number of diabetics increased from 108 million in 1980 to 422 million in 2014. Currently, the number of diabetics in the world is estimated more than 220 million, and the number of deaths will double between 2005 and 2030 [2]. Diabetes mellitus is divided into type 1 and 2 diabetes, and other less common forms such as maturity-onset diabetes of the young [3], genetic defects in insulin action, and the antagonism of growth hormone as gestational diabetes mellitus [4]. There are several chemically induced animal models of type 1 diabetes. One of the most common chemicals used to generate type 1 diabetes animals

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is alloxan [5]. Alloxan is a well-known and universally used agent for evoking experimental diabetes through its toxic effect on the B cells of the Langerhans islets. which can subsequently lead to hyperglycemia within a few days [6]. The dose required to induce diabetes with these chemicals depends on the animal species, route of administration, and nutritional status [5].

Alloxan (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetrone is oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution [7]. It is an acidic compound formed by oxidation of uric acid. Currently, alloxan is added to food materials, especially to the all-purpose flour (maida) to bring softness and white color to the flour [8]. Hence, consuming foodstuffs made from this flour can lead to diabetes, because alloxan tampers with the pancreas and aggravates already existing diabetes or increases the risk of developing diabetes a, making it imperative to develop an accurate estimation of alloxan in food items by voltametric-based technique [9].

Alloxan is a toxic glucose analogue (in the shape and hydrophilicity), which selectively destroys insulin-producing β-cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus called alloxan diabetes in these animals, with characteristics similar to type 1 diabetes in humans [10, 11]. The toxic effect of alloxan on pancreatic beta cells includes oxidation of basic sulfhydryl (-SH) groups (because it is a weak acid) of intracellular thiols and, as a result, reduction of glutathione content, redox reactions that generate reactive oxygen species (ROS), diauric acid, and inhibition of glucokinase, a protein containing SH is necessary for glucose-induced insulin secretion and disturbances in intracellular calcium homeostasis, **Figure 1**, [11, 13]. Thus, this study aimed to evaluate and measure the alloxan concentration in blood samples of Libyan children with type 1 diabetes.

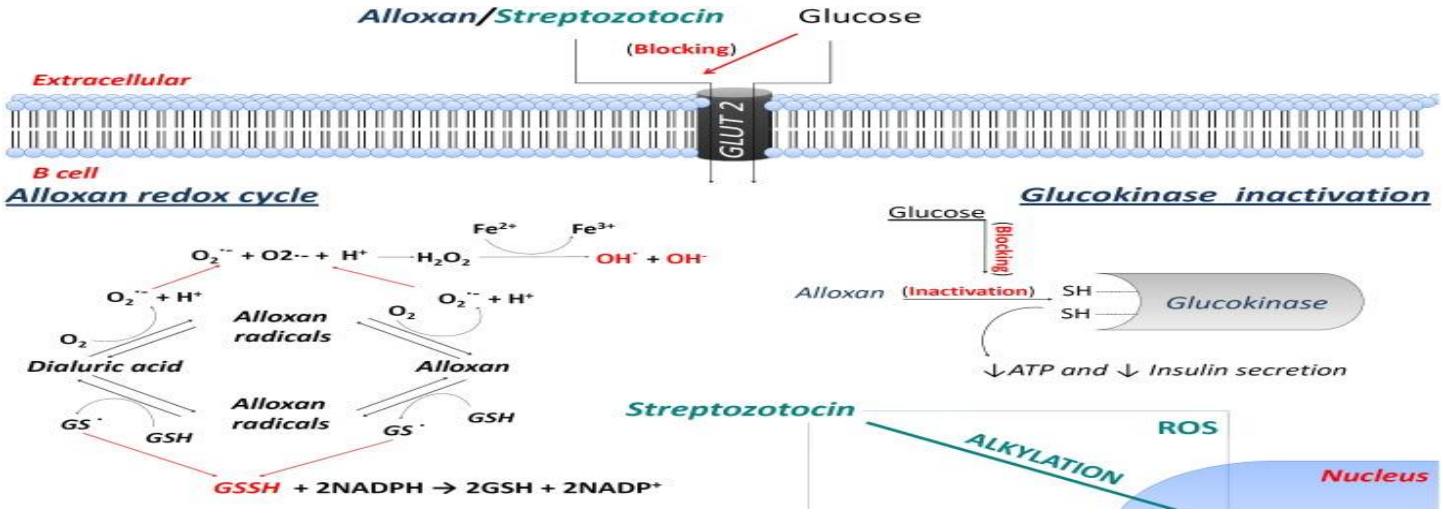


**Figure 2:** Mechanism of action of alloxan monohydrate in beta cells

## Materials and methods

*Volunteers:* 45 healthy Libyan children ages between 5-15 years (10.0±5.0 years) in this study. 15 healthy and 30 patients with diabetes mellitus (females: 53.0%, males: 47.0%). The samples collected between (July-November, 2024) from Tripoli Medical Hospital, Tripoli-Libya. Volunteers with a history of systemic diseases such as thyroid disorder and kidney were excluded. The children under study with insulin-dependent diabetes mellitus is treating with insulin at doses in the range (4-24 U/kg body-weight).

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*Sample collection:* Diagnostic samples with diabetes mellitus were gathered from the endocrinology department of Tripoli Medical Hospital, in the other hand, healthy samples from relatives. The questionnaire was filled out by volunteers (healthy and diabetic), and blood sample was collected in glass tubes from each volunteer.

*Sample analysis:* Venous blood was taken from the elbow vein and transferred immediately into deproteinization solution avoiding any contact with oxygen. The concentration of alloxan in blood samples determined by Archibald spectrophotometric method [14].

*Statistical analysis:* All statistical analysis was done using IBM SPSS Version 21 program. Mean±S.D and median of data was calculated using descriptive analysis. A comparative analysis between groups was done by using Mann-Whitney U-test. Differences are considered statistically significant at $p<0.05$.

## Results

The determination of endogenous alloxan content in blood of healthy donors it was stabilized by rapid lowering pH=2.0. Alloxan was then allowed to form a colored product in a reaction with (o-phenylenediamine) and its content was measured by spectrophotometry using an internal calibration curve. Analysis of 75 blood samples of healthy volunteers has shown that in most donors alloxan concentrations vary from 41 to 265 µmol/L. However, in a small group of healthy people alloxan levels greatly exceeded the above-mentioned limit [15]. In the current study, alloxan was estimated in the blood of children with type 1 diabetes using the spectrophotometric method, using an internal calibration curve ($y=0.0001x-0.0006$, $R^2=0.9949$). The 45 volunteers were divided into healthy volunteers (n=15) and diabetic patients (n=30).

*Alloxan concentration in blood samples of children with diabetes mellitus and healthy:* The study evaluated the alloxan concentration in 15 healthy, 30 diabetic patients. Overall results are summarized in **Figures 3** and **4**. The result showed a highly variation of alloxan level between patient (0.36-6.126 mg/ml) and healthy volunteers (0.036-1.726 mg/ml) in blood, statistically with high significant ($p<0.05$).

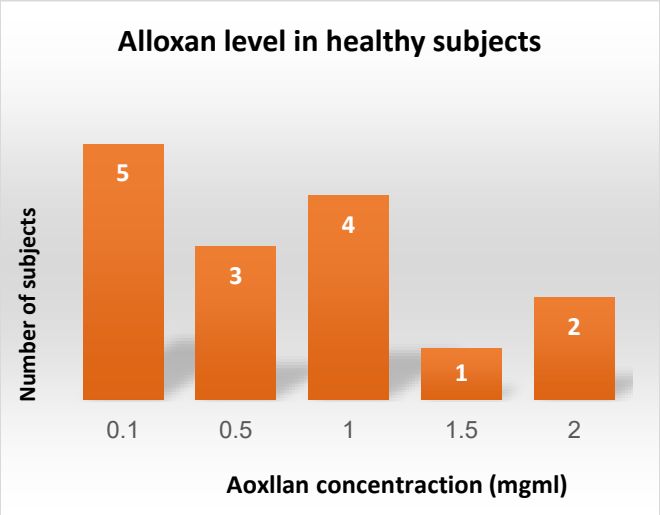


**Figure 3:** Alloxan level in healthy subjects

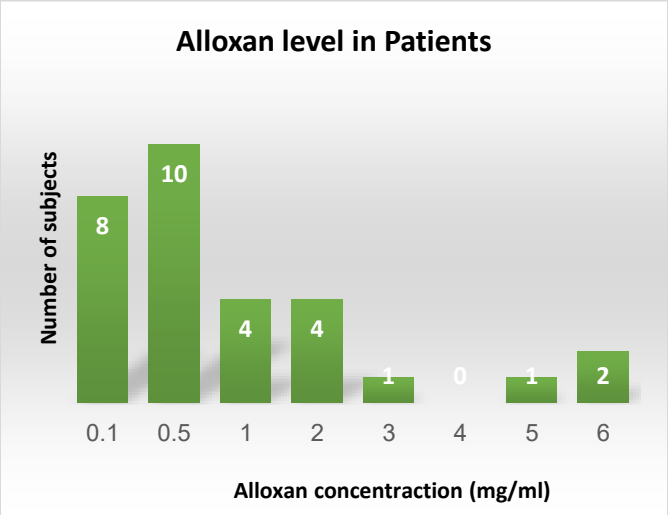


**Figure 4:** Alloxan level in patient subjects

According to the questionnaire of diabetes mellitus children, the volunteers divided into three groups based on diet care **Table 1**.

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**Table 1:** The divided groups of diabetes mellitus children based on diet care

| No. | Group | Frequency | Total D.M. patient under study |
|---|---|---|---|
| **1** | Low-carb diet low adherence diabetics group | 14 | 46.6% |
| **2** | Low-carb diet moderate adherence diabetics | 8 | 26.6% |
| **3** | low-carb diet high adherence diabetics | 8 | 26.6% |

*Healthy and low, mediate and high carbohydrate diet low adherence of children with diabetes:* The alloxan concentration of healthy group is (0.66-1.725 mg/ml, median=0.458) versus to the low c carbohydrate arb diet low adherence was (0.326-6.236 mg/ml) in blood samples with median=1.91, statistically showed difference of significant. For low carbohydrate diet high adherence was (0.036-0.556 mg/ml) with median=0.101, either low carbohydrate diet high adherence with significant. In contrast, low carbohydrate diet moderate adherence (0.076-2.406 mg/ml of alloxan concentrations) showed insignificant in comparing with healthy subjects **Table 2**.

**Table 2:** Median of healthy and low-carbohydrate diet low adherence diabetics group

| Group | Frequency | Median (MD) |
|---|---|---|
| **Healthy group** | 15 | 0.458 |
| **Low-carbohydrate diet low adherence diabetics group** | 14 | 1.91 |
| **Low-carbohydrate diet moderate adherence diabetics** | 8 | 0.89 |
| **low-carbohydrate diet high adherence diabetics** | 8 | 0.101 |

Alloxan level in low carbohydrate diet high adherence lower than in healthy individuals. This, in turn, may affect their health in terms of excessive obesity and increased formation of ROS in large quantities within the body, which affects the functioning of the pancreas, and the condition may worsen to the diabetes development.

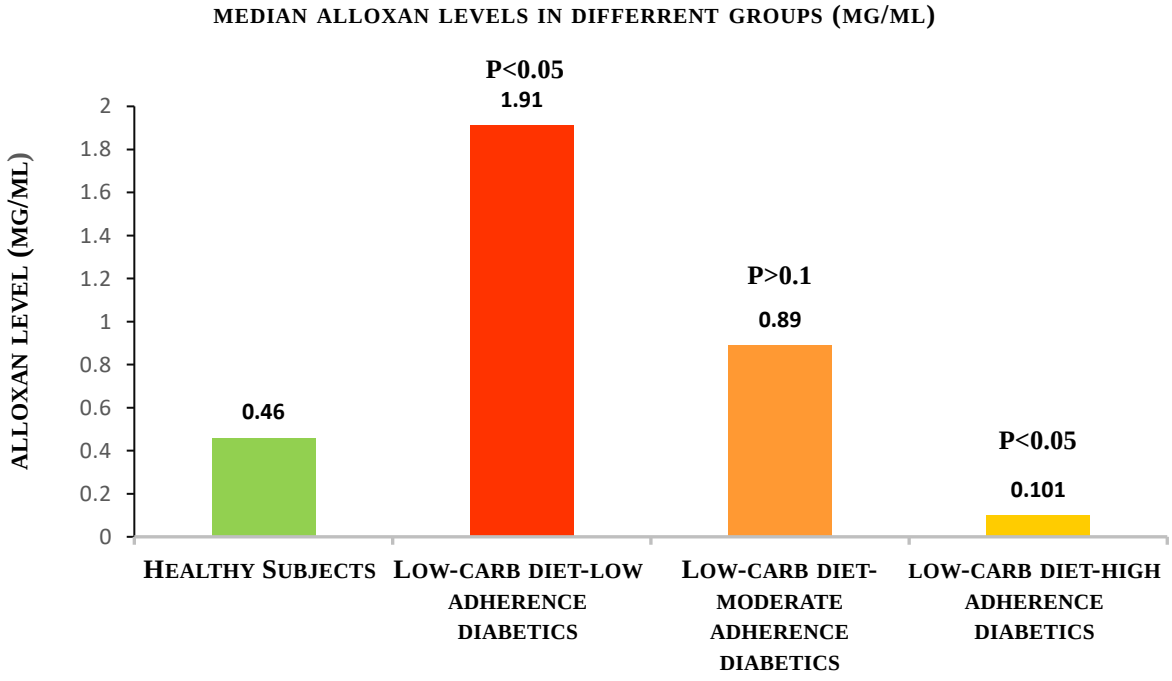


**Figure 5:** Median alloxan concentration levels in different groups

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In **Figuer 5**, the groups with diabetes mellitus of carbohydrate diet- between low and high adherence showed highly significant of alloxan level in the blood, with lowest value in low-carbohydrate diet-high adherence group (0.10 mg/ml) with a clear difference, statistically significant (P<0.001). As illustrated in **Figure 5**, the differncy of alloxan level in blood between carbohydrate diet-moderate and high adherence with a difference statistically highly significant of alloxan level in blood, with lowest value (under 90.0%) in high-carbohydrate diet-high adherence group (0.10 mg/ml). In the other side, there is no significant of alloxan level in blood samples between carbohydrate diet-moderate and low adherence groups.

## Discussion

Patients with type 1 diabetes mellitus experience high fluctuations in the blood glucose levels and difficulties in achieving glycemic goals (glycated hemoglobin ≥7.0% or ≥353 mmol/mol), which increases risk of developing many acute and chronic health complications including cardiovascular disease [16-19]. Dietary carbohydrates significantly affect postprandial blood glucose levels, and the National Health and Medical Research Council recommends that individuals with type 1 diabetes follow a low-carbohydrate diet (HC: 45.0-65.0% of total energy intake) [20]. Understanding the mechanism of action of typical diabetogenic agents is important for elucidating the causes of diabetes. The discovery that alloxan present in humans blood led us to evaluate the level of alloxan in blood from patients with insulin-dependent diabetes. In previous studies to estimate alloxan in the blood of people with diabetes, especially children with Type 1, it was estimated spectrophotometrically in 68 children aged 6-15 years and in a control group of 44 healthy children in the same age range. The mean level of alloxan in blood from children with insulin-dependent diabetes mellitus was 8.76±9.64 µg/ml and in blood from healthy children was 1.53± ~ .10 µg/ml [21]. Alloxan levels in a diabetic group with moderate adherence to a low-carbohydrate diet comparing them with alloxan levels in healthy subjects, the test revealed a non-significant difference in alloxan levels. On the other hand, by comparing the levels of alloxan in the group of healthy volunteers and the group of diabetics following a low-carbohydrate diet, the test revealed a significant difference of alloxan levels, **Figure 5**. Alloxan is sometimes used in the manufacture of dyes, but its main use is to induce diabetes in laboratory rodents. Fortunately, alloxan it has toxicity to the liver and kidneys, and leads to acute tubulointerstitial nephritis, which subsequently leads to nephrotoxicity [22]. Renal and hepatic toxicity resulting from alloxan will generate a ROS when these bodies accumulate ROS, it will lead to cell damage, and these cells may be pancreatic cells, especially beta cells, and recently lead to their death, the occurrence of diabetes, and the development of complications. The determination of alloxan in the blood is not limited only to diabetic volunteers, but even to healthy volunteers, because alloxan enters the body through the consumption of bleached flour used in pastries. The results showed that the concentration of alloxan was higher in healthy people compared to diabetic volunteers who follow a low-carbohydrate diet, they reducing their consumption of pastries as much as possible. Previous study showed that high concentrations of alloxan can appear in healthy volunteers who do not suffer from type 1 diabetes mellitus [21].

*Conclusion:* Healthy children with high alloxan blood level must be receiving medical attention. Children with diabetes should follow a low-carbohydrate diet to be prevented from development of complications.

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**Ethical issues:** The authors completely observed ethical issues including plagiarism, informed consent, data fabrication or falsification, and double publication or submission.
**Data availability statement:** The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
**Author declarations:** The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.