Original article:
Elevated Plasma and Liver tissue Uric Acid Levels in Alloxan Diabetic Rats
Yogaraje Gowda C V1, Senthilkumar S2, Kashinath R T3

Abstract:
Background: Abnormal levels of serum uric acid (UA) causes major health problem due to its pivotal role in the etiology of many systemic diseases. Many research works in recent past have shown elevated uric acid levels in diabetic subjects. Some reports indicate that uric acid elevation is related to diabetic complications, whereas a few claimed that uric acid elevation is also seen in pre-diabetic condition. The reason for this elevation of uric acid and a possible role of insulin in this regard is obscure. Hence a study has been undertaken to assess the uric acid status in alloxan diabetic rats with an attempt to establish the possible cause for uric acid elevation. Methods: The studies were carried out on healthy male Wistar rats with a body weight of 150-180g. The rats were divided into two groups, normal group (Group-1) and alloxan diabetic group (Group-2) with six animals in each group. Induction of diabetes was done by administering a single intraperitoneal injection of freshly prepared aqueous solution of Alloxan Monohydrate (150mg/Kg body weight) prepared in normal saline, to the overnight fasted rats. After the stipulated period of 30 days, the animals (Group-1 and Group-2) were anesthetized using Isoflurane and sacrificed. They were dissected immediately and liver tissue was procured, blotted to remove blood stains, and placed in cold phosphate buffer saline (pH7.4). Blood samples were collected using heparin as anticoagulant. The uric acid levels in plasma, erythrocytes and in liver tissue as well as the levels of ADA in plasma and liver tissue were estimated. Results: A significant (p<0.001) rise in uric acid levels in plasma, erythrocytes and liver tissue as well as increased levels of ADA in plasma and liver tissue was observed in alloxan diabetic rats compared to normal control. Conclusion: The increased uric acid levels noticed in alloxan diabetic rats may be due to increased catabolism of purines as evidenced by increased activity of ADA.

Keywords: Alloxan diabetes; plasma uric acid;liver tissue uric acid; ADA

Introduction:
Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism due to absolute or relative deficiencies in insulin secretion or insulin action or both. The latest IDF (International Diabetes Federation) estimates indicate that 415 million (1 in 11 persons) have diabetes, and this will increase to 642 million or almost 10% of the general population by 2040. Following our earlier report, several researchers have claimed that uric acid, the final oxidation product of purine catabolism is elevated in diabetic subjects. Despite enormous research activities, the prevalence of diabetic related complications is increasing worldwide. Some reports indicate that uric acid elevation is related to diabetic complications involving retina, kidneys, nerves and cardiovascular system whereas a few claimed that uric acid

1. Mr. Yogaraje Gowda CV, Lecturer in Biochemistry, Bangalore Medical College & Research Institute, Bengaluru-560 002, Karnataka, India and Research Scholar, Department of Research and Development, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandlam, Chennai-602 105, India.
2. Dr. Senthilkumar S, Associate Professor, Department of Research and Development, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandlam, Chennai-602 105, India.
3. Dr. Kashinath R T, Professor, Department of Biochemistry, Director, Department of Research and Development, Subbaiah Institute of Medical Sciences, Purle, Shivamogga- 577 222, Karnataka, India.

Correspondence to: Mr. C V Yogaraje Gowda, Lecturer in Biochemistry, Bangalore Medical College & Research Institute, Bengaluru-560 002, Karnataka, India. Email: cvyogaraja@gmail.com.
Elevation is seen in pre-diabetic condition. The reason for this elevation of uric acid and a possible role of insulin in this regard is obscure. Hence a study has been undertaken to assess the uric acid status in alloxan diabetic rats. Also an attempt has been made to establish the possible cause for uric acid elevation.

**Materials and methods**

**Chemicals:**
Alloxan monohydrate was procured from Sigma Aldrich chemicals (St. Louis, U.S.A) and other chemicals used were of Analytical Reagent(AR) grade, purchased from Randox Laboratories (UK).

**Animals:**
The studies were carried out on healthy male albino Wistar rats (body weight of 150-180g) which were randomly selected and housed in polycarbonate cages under normal 12 hours day-night cycle and with temperature maintenance of 22±2°C. The rats were fed with commercial rat feed (Amruth Rat Feed, supplied by Pranav Agro Industries, Pune, India) and had free access to water *ad libitum*.

The animal experiments were conducted as per CPCSEA guidelines and as per the norms of Institutional Animal Ethics Committee (IAEC).

**Statistical Evaluation:**
The data entry was carried out using Microsoft Office Excel worksheet and statistically analyzed. The results were expressed as mean ±SD. The p value was calculated by Student ‘t’ test.

**Induction of Diabetes Mellitus:**
Diabetes was induced by administering a single intraperitoneal injection of freshly prepared aqueous solution of Alloxan Monohydrate (150mg/Kg body weight) (3,4) in normal saline, to the overnight fasted rats. The onset of diabetes was monitored 48 hours after alloxan injection. Rats with blood-glucose levels above 250mg/dL were considered diabetic and were employed in the current study.

**Animal Groups:**
The experimental rats were divided into two groups with six animals in each group.

1) **Group-1 (Normal group):** Consisted of six normal rats which were maintained on commercial rat pellet feed and water *ad libitum*

2) **Group-2 (Alloxan diabetic group):** Consisted of six alloxan diabetic rats. These rats were maintained on commercial rat pellet feed and water *ad libitum*.

After a stipulated period of 30 days, the animals were anesthetized using isoﬂurane, and blood was collected in heparinised tubes by retro-orbital puncture. The rats were weighed and sacrificed by injecting with sodium pentobarbitone. They were dissected immediately and the blood stains were removed by blotting the liver tissue, and placed in cold phosphate buffer saline (pH7.4). The isolated liver tissues of both normal and alloxan diabetic groups were cut into small pieces of 1.0g each.

Blood plasma was separated by centrifugation at 3000rpm for 6-8 min, 8°C. The clear plasma was then used for the estimation of uric acid and ADA levels. The sedimented erythrocytes were washed 4 times with 5mL aliquots of normal saline. The washed erythrocytes were suspended in normal saline so as to give a 50% erythrocyte saturation. 1mL of this 50% saturated erythrocyte suspension was mixed with 4mL 10%TCA to remove proteins.

After 10 min, the tubes were centrifuged at 3000 rpm for 6-8min, 8°C and the clear supernatant was used for uric acid estimation. The liver tissue was mixed with phosphate buffer pH7.4 (1:9) and homogenized. The homogenate was centrifuged at 3000 rpm for 6-8min, 8°C and the clear supernatant obtained was employed for the estimation of uric acid and ADA levels.

**Ethical clearance:** This study was approved by institutional Ethics committee. (BMCH/IAEC/05 Biochem/2015, dated 04.06.2015)

**Results:**
The results of the present study are depicted in Table-1 and Table-2.

Table-1, Figure -1 and Figure-2 depicts plasma glucose levels, body weight, liver weight, ratio of body weight to liver weight in normal and alloxan diabetic rats. The results indicate a significant rise in the levels of plasma glucose in alloxan diabetic rats (Group-2) compared to normal rats (Group-1). A significant (p<0.001) increase in body weight in Group-2 as compared to Group-1 was observed, whereas the somatic ratio doesn’t show much variation.
Table-2, Figure -3 and Figure-4 reveals uric acid levels in plasma, erythrocytes and liver tissue homogenates as well as ADA levels in plasma and liver tissue homogenates of Group-1 and Group-2 rats.

The results indicate that uric acid levels are significantly (p<0.001) elevated in plasma, erythrocytes and liver tissue homogenates of alloxan diabetic rats (Group-2) compared to normal rats (Group-1). Further, table-2 reveals that ADA levels in both plasma and liver tissues are significantly (p<0.001) elevated in Group-2 rats compared to control rats.

**TABLE-1**

Table-1 shows plasma glucose level, body weight, liver weight and ratio of body weight to liver weight in normal as well as in alloxan diabetic rats.

| Groups            | Plasma glucose (mg/dL) | Initial body weight in g | End body weight in g | % of body weight | Liver weight in g | End body weight/liver weight |
|-------------------|------------------------|--------------------------|----------------------|------------------|-------------------|-----------------------------|
| Group-1 (n=6)    | 82.3 ± 10.13           | 152 ± 1.63               | 174.5 ± 14.63        | 13.31 ± 1.51     | 4.4 ± 0.95        | 40.7 ± 7.07                 |
| (Normal)         |                        |                          |                      |                  |                   |                             |
| Group-2 (n=6)    | 176.5 ± 22.98***       | 175 ± 2.60***            | 291.5 ± 30.81***     | 39.93 ± 0.96***  | 6.6 ± 0.64***     | 44.28 ± 6.96                |
| (Alloxan diabetic)|                        |                          |                      |                  |                   |                             |

Note:
1. Number in parenthesis indicate the number of rats
2. The values are expressed as their mean ± SD
3. Statistical evaluation-probability level* p< 0.05, ** p<0.01, *** p<0.001.

**Discussion:**

Uric acid (UA) (2,6,8-trihydroxypurine,C$_5$H$_6$N$_4$O$_3$) a heterocyclic weak organic acid, formed during the oxidation of purine nucleotides in humans. The purine nucleosides and purine nucleotides are catabolised to uric acid through the enzymes ADA and 5’ nucleotidase. Several researchers have reported elevated uric acid levels (i.e. hyperuricaemia) in type-2 diabetic subjects and the elevation is more significant in diabetic complications. ADA is a polymorphic enzyme, which catalyses the
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Figure 3: The graph depicts the plasma, liver and erythrocyte uric acid levels. Results are expressed as Mean ± SD (n =6); ***P < 0.001 as compared to normal control group.

Figure 4: The graph depicts the plasma and liver ADA levels. Results are expressed as Mean ± SD (n =6); ***P < 0.001 as compared to normal control group.

Irreversible deamination of adenosine to inosine and thus involved in the regulation of intracellular and extracellular concentration of adenosine.

The elevated plasma uric acid levels observed in the present study in alloxan diabetic rats is in agreement with previous reports which have indicated an increment in the level of uric acid in diabetes mellitus4-11. A parallel raise in liver tissue uric acid levels (Table-2, Figure-3) in alloxan diabetic rats suggests that the increase in plasma uric acid levels may be due to increased formation of uric acid in liver. A significant elevation in liver tissue ADA activity in alloxan diabetic rats(Table-2, Figure-4) further suggests increased formation of uric acid by purine breakdown in alloxan diabetic liver.

Adenosine plays an important role in the regulation of many metabolic processes including hormone release, and has been reported as a potent antagonist of insulin action in various tissues 21.

ADA, apart from being involved in adenosine degradation is also responsible for decreasing the tissue levels of adenosine, and hence the tissue adenosine levels are related to total glucose output of the tissue21.

Tissues by reducing the cellular content of adenosine probably through an increase in the catabolism of adenosine by ADA may favor insulin action as it is known that adenosine antagonizes insulin action19,20. This action of tissue cells of reducing cellular adenosine levels through raised ADA activity results in increased nucleoside breakdown and may result in increased uric acid production as seen from the results obtained in the present studies.

Conclusion: It can be concluded that a significant elevation of uric acid levels in plasma, erythrocytes and liver tissue of alloxan diabetic rats, is due to increased degradation of tissue adenosine through an increased activity of ADA in order to facilitate the available insulin action as adenosine is an antagonist of insulin action.

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Author’s contribution:
Data gathering and idea owner of this study: C V Yogaraje Gowda and R T Kashinath
Study design: R T Kashinath, C V Yogaraje Gowda and S Senthilkumar
Data gathering: C V Yogaraje Gowda
Writing and submitting manuscript: C V Yogaraje Gowda
Editing and approval of the final draft: C V Yogaraje Gowda, S Senthilkumar
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