Modulation of purine metabolism and uric acid level by gut microbiota product, Acetate; in Streptozotocin-induced diabetes mellitus in male wistar rats

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Abstract

Sodium acetate is a gut microbiota product which has shown potential metabolic benefits on several disease models. Several studies have revealed that gut microbiota products enhance insulin sensitivity, improve glucose uptake and suppress cardiometabolic alterations in Diabetes Mellitus (DM). However, acetate influence on purine metabolism in DM has not been fully explored. The aim this study was to investigate the effect of acetate on blood glucose level, insulin sensitivity, purine metabolism and uric acid level in streptozotocin-induced diabetes. A total of thirty (30) male Wistar rats of about 8-10 weeks weighting between 140g-220g were used for the study. Group 1: control. Group 2: Diabetic (untreated). Group 3: sodium acetate (200 mg/kg orally). Group 4: Metformin group (100 mg/kg orally). Group 5: Diabetic with sodium acetate (200 mg/kg). Group 6: Diabetic + metformin (100 mg/kg). All groups were treated for 21 days (n= 5/group). The findings from this study showed that sodium acetate enhanced insulin sensitivity, xanthine oxidase/adenosine deaminase activities and uric acid level ($p< 0.05$) in comparison to the diabetic control rats. The results of this study suggest the beneficial effect of acetate by modulating purine metabolism via adenosine deaminase/xanthine oxidase and uric acid pathway in diabetes mellitus.

Keywords: Acetate, Purine metabolism, Uric acid, Diabetes mellitus

Introduction

Purine metabolism consists of three main interconnected pathways which includes: de novo synthesis, catabolism and salvage pathways. Aside being known as building blocks for DNA and RNA, purines also provide cell with the necessary energy and co factors to promote cell survival and

proliferation. There is a renew interest in how purine metabolism may aggregate metabolic disorder such as diabetic complication through metabolic organization. Guanine is converted to xanthine via xanthine oxidase-oxidation of hypoxanthine whereas in the cells, adenosine deaminase (ADA) converts adenosine to inosine and xanthine oxidase (XO) converts hypoxanthine to xanthine and then to uric acid. Elucidating the regulation of purine metabolism through these enzymes may provide a potential target for treatment of diabetics and prevent progressive complication (Varadajah *et al.,* 2022, Masato 2020, Anthony and Stephen, 2017). Targeting the purine metabolism salvage and not just uric acid will prevent metabolic disease as well as reduce cardiovascular morbidity (Masato, 2020).

Adenosine deaminase (ADA) is a polymorphic purine enzyme, and the activity has been demonstrated to initiate response via its ability to reduce adenosine (Adewumi 2020, Xu *et al.,* 2017). It clears adenosine by converting it to inosine via an irreversible deamination reaction. ADA also stimulates immune responses in tissues (Cristalli *et al.,* 2001) and its activity has been reported to be relatively high in the skeletal muscle, liver, fatty and lymphoid tissues (Niedzwicki and Abernethy, 1991). Likewise elevated ADA activity had been reported in patients with type 2 diabetes mellitus (T2DM) (Kurtul *et al.,* 2005). The levels of adenonosine, xanthine and uric acid may be useful for monitoring the progression of diabetics (Xia, 2024; Cristalli *et al.,* 2001). The activities of ADA and XO can influence uric acid levels, therefore changes in these enzyme activities may be associated with various metabolic conditions such as insulin resistance and oxidative stress-related diseases. Sodium acetate on the other hand has been shown to protect the liver gestational metabolic alterations by inhibiting uric acid and lactate production indicating that it could as well modulate purine metabolism (Adewumi *et al.,* 2020, Tolulope, 2019).

Xanthine oxidase (XO) catalyzed the penultimate and final steps of purine metabolism, which produce uric acid from hypoxanthine (Mandal and Mount, 2015). XO is most expressed in liver and gut (Saksela, Lapatto and Raivio). XO engages molecular oxygen as electron acceptor and with this generates superoxide anion and other reactive oxygen species as by-product. Reactive oxygen species (ROS) promotes oxidative stress related tissue injury which consequently converts to XO and uric acid (Kelley *et al.,* 2010). Increased plasma XO activity is associated with obesity, smoking,

liver deregulation, hyperuricemia, dyslipidemia, and insulin resistance (Masato, 2020). This has made hyperuricemia to be thought as a surrogate marker of metabolic syndrome (Du *et al*., 2024). Plasma uric acid level has been reported to correlate linearly with indices of insulin resistance such as homeostatic model assessment of insulin resistance (HOMA-IR) (Sumito *et al.,* 2018).

In recent years attention has been drawn on the state of purine metabolism (PM) in diabetes mellitus and the possible role or disturbances in the metabolism of uric acid (UA) in the manifestation and progression of this endocrine pathology. Imbalance of UA has been proven to be a risk factor for the occurrence of conditions associated with diabetes mellitus such as cardiovascular disease, hypertension, kidney diseases and vascular injury (Cherniaieva *et al.,* 2018). Other studies also showed that normalizing dysfunctional purine metabolism accelerates diabetic wound healing (Weinstein, 2015). It is believed that purine metabolism pathways regulate insulin secretin and glucose metabolism, consequently, they are involved in the pathological mechanisms of T2DM development (Alisan *et al.,* 2022).

Several studies have shown the relationship between PM with metabolic syndrome and type 2 diabetes however; more research is still required to establish the significance of PM in diabetes. Therefore, the aim of this research was to elucidate the modulating effect of acetate a gut microbiota product on purine metabolism and uric acid level in diabetes.

Research methodology

This study was conducted using 30 male Wistar rats, each weighing approximately 200–250 grams. The rats were housed at the animal house College of Health Sciences, Prince Abubakar Audu University, Anyigba, Kogi State under standard laboratory conditions with a 12-hour light/dark cycle and free access to food and water. The animals were allowed to acclimatize for one week before the experiment. The rats were then randomly divided into six groups (n=5 per group) (Table 1). All experimental protocols were in accordance to College of Health Science research ethical committee (CHSREC) CREC-CHS/PAAU/2025/0004.

Table 1: Animal grouping and their treatment

Groups/ n=5	Treatment	Dosage/ *po*
Control group (CTR)	Distilled water	10ml/kg
Diabetic group (DIA)	Diabetic with Distill water	10ml/kg
Sodium acetate group (ACE)	Non-diabetic rats treated with sodium acetate	200 mg/kg
Metformin group (MET)	Non-diabetic rats treated with metformin	100 mg/kg
Diabetes + Metformin group (DIA+MET)	Diabetic rats treated with metformin	100 mg/kg
Diabetes + Sodium acetate group (DIA+ACE	Diabetic rats treated with sodium acetate	200 mg/kg

All treatments lasted for 21 days.

Induction of Diabetes and **Blood glucose testing:** Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (Millipore Sigma, St. Louis, Mo., USA) at a dose of 65 mg/kg after an overnight fast (Ghasemi *et al.,* 2023). After 72 hours of STZ injection, the blood glucose levels were estimated using a glucometer (Fine test®) kit. The tip of each rat's tail was gently pricked using a sterile needle to transfer a drop of blood to the test strip. Rats with blood glucose levels above 200 mg/dL were considered diabetic and included in the study. (Ghasemi *et al.,* 2023).

Estimation of Fasting Blood Glucose (FBG): FBG was monitored on days 7, 14, and 21 respectively (Ben *et al*, 2023). FBG was determined after 12 hours over night fast.

Estimation of Insulin Levels: Measured by enzyme-linked immunosorbent assay (ELISA). These assays utilize antibodies specific to insulin, which bind to the insulin in the sample. The bound insulin is then detected using a labeled antibody, allowing for quantification of the insulin concentration.

Determination of indices of insulin resistance HOMA-IR (Homeostatic Model Assessment of Insulin Resistance): HOMA-IR was calculated using fasting glucose and insulin levels with the formula:

$$\text{HOMA-IR} = \frac{\text{Fasting insulin (U/mL)} \times \text{fasting glucose(mg/dL)}}{405}$$

Estimation of Xanthine oxidase activity: XO catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid, generating hydrogen peroxide (H2O2). XO is estimated using colorimetric or spectrophotometric methods. In this method, XO activity is measured by monitoring the rate of uric acid production or the consumption of hypoxanthine, often using colorimetric or spectrophotometric methods (Sögüt et al., 2002)

Adenosine Deaminase (ADA) Estimation: ADA activity was measured by monitoring the rate of hypoxanthine formation or the

consumption of adenosine, often using colorimetric or spectrophotometric method. ADA catalyzes the conversion of adenosine to inosine, which is then converted to hypoxanthine by purine nucleoside phosphorylase (PNP). (Sögüt et al., 2002)

Uric Acid Estimation: Uric acid was measured using enzymatic methods (Badmus *et al.,* 2021), where it was estimated as the end product of purine metabolism using reagents obtained from Fortress Diagnostics Limited, Antrim, UK

Data analysis and statistics

All data were expressed as means ± SEM. Statistical analysis was performed with SPSS statistical software 22.0. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparisons of mean values between the groups. Statistically significant differences were accepted at P <0.05

Results

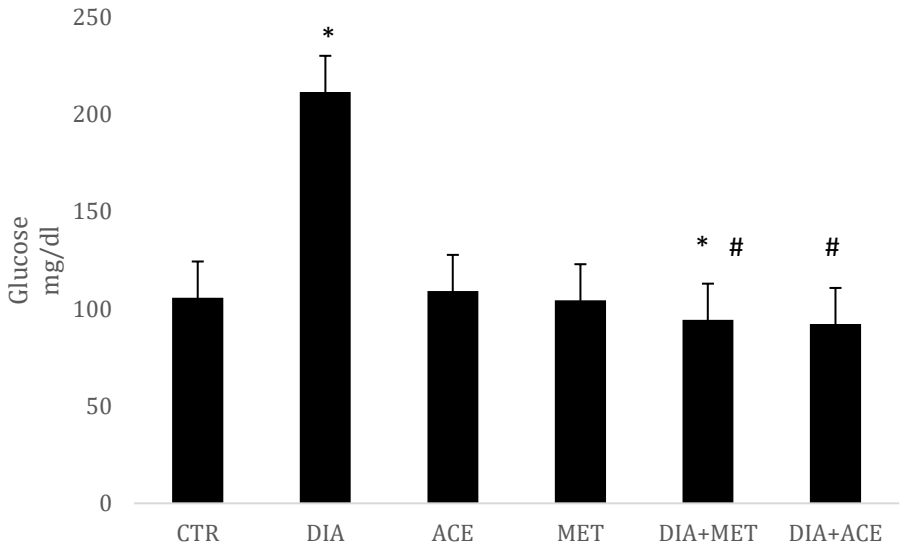
Effect of Acetate on Blood Glucose and Insulin

The result showed that there was significant increase in glucose level in diabetic untreated group compared to control group (< 0.05) however, glucose level significantly decreased in the diabetic with acetate treated group compared to the diabetic untreated group (< 0.05) (Figure 1). Also, the result showed significant decrease in insulin level of the diabetic untreated and diabetic with acetate treated groups compared to the control (< 0.05) (Figure 1)

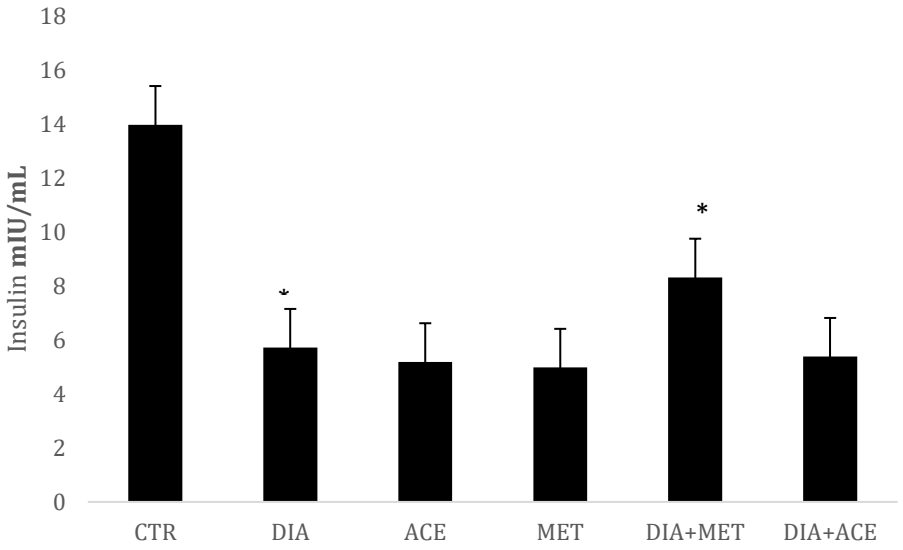
Effect on Xanthine oxidase, Adenosine deaminase and Uric acid level

The result showed significant increase in adenosine deaminase and xanthine oxidase activities in the diabetic untreated group compared to the control group (< 0.05) however, diabetic with acetate group showed significant decrease in adenosine deaminase and xanthine oxidase activities when compared to the diabetic untreated group (< 0.05) (figure 2). In addition, there was significant increase in uric acid level in diabetic untreated group compared to the control group however, diabetic with acetate treatment showed significant decrease in uric acid level compared to the diabetic group (figure 2).

(a)



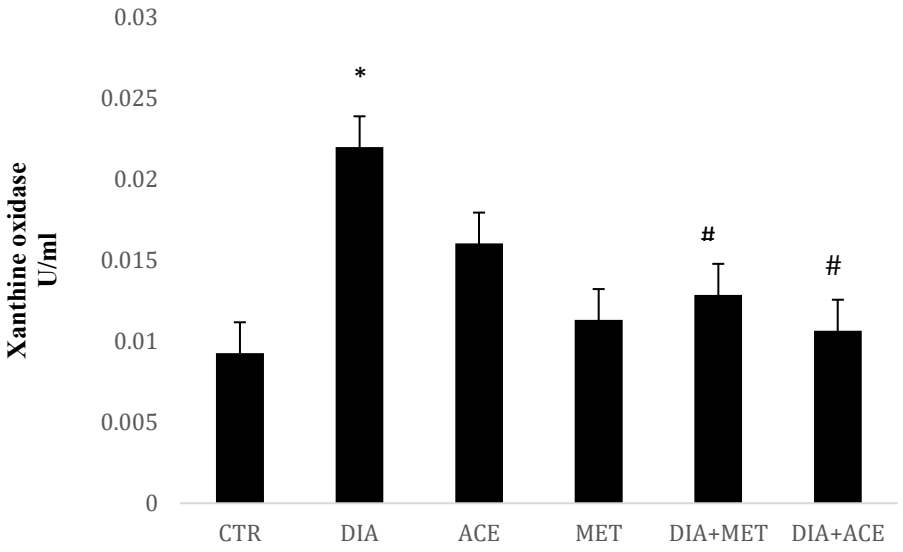
CTR=control, DIA= Diabetic untreated, ACE = Acetate, Met = metformin, Dia+Met= Diabetic treated with metformin, DIA+ACE = Diabetic treated with acetate



CTR=control, DIA= Diabetic untreated, ACE = Acetate, Met = metformin, Dia+Met= Diabetic treated with metformin, DIA+ACE = Diabetic treated with acetate

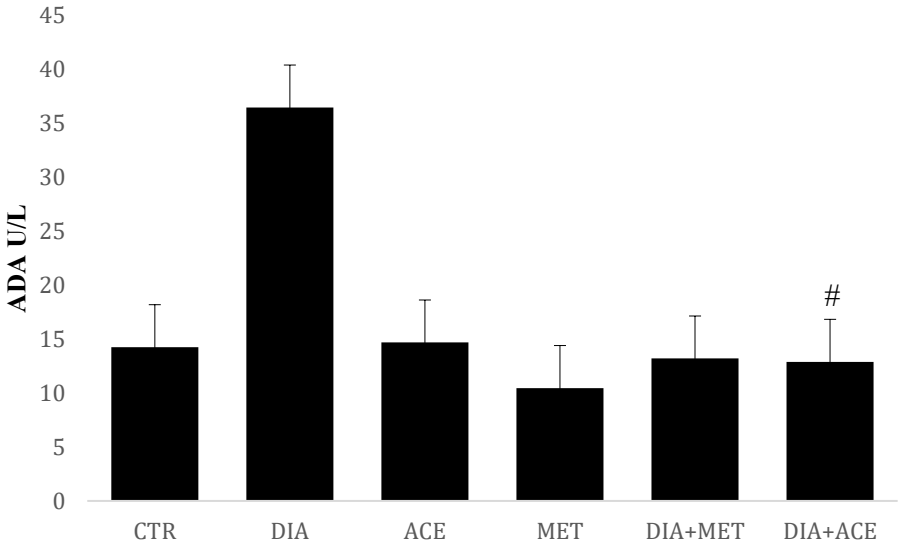
Figure 1: Effect of Acetate on (a) blood glucose and (b) insulin in streptozoccin-induced diabetic rats. There was significant increase in blood glucose but decreased insulin level of diabetic rats compared to the control whereas diabetic plus acetate treated rats showed significant decrease in blood glucose. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group. * DIA VS CTR $P<0.05$ # DIA+ACE VS DIA $P<0.05$

(a)

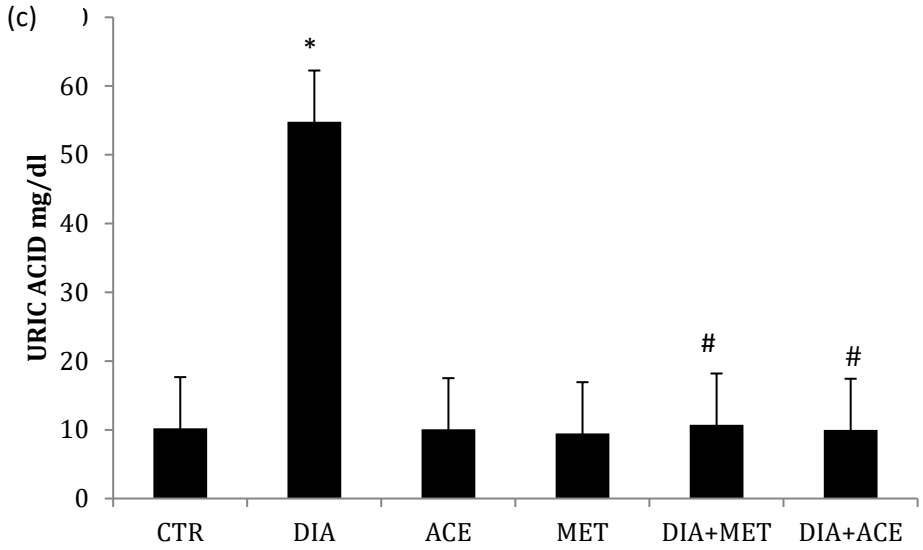


CTR=control, DIA= Diabetic untreated, ACE = Acetate, Met = metformin, Dia+Met= Diabetic treated with metformin, DIA+ACE = Diabetic treated with acetate

(b)



CTR=control, DIA= Diabetic untreated, ACE = Acetate, Met = metformin, Dia+Met= Diabetic treated with metformin, DIA+ACE = Diabetic treated with acetate



CTR=control, DIA= Diabetic untreated, ACE = Acetate, Met = metformin, Dia+Met= Diabetic treated with metformin, DIA+ACE = Diabetic treated with acetate

Figure 2: Effect of Acetate on (a) xanthine oxidase, (b) adenosine diaminase and (c) uric acid in streptozoccin-induced diabetic rats. There was significant increase in xanthine oxidase, adenosine and uric acid of diabetic rats compared to the control whereas diabetic plus acetate treated rats showed significant decrease in xanthine oxidase, adenosine and uric acid. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group. * DIA VS CTR $P<0.05$ # DIA+ACE VS DIA $P<0.05$

Discussion

The present study demonstrated diabetes mellitus in male Wistar rats showing elevated fasting blood glucose, but a decrease in fasting insulin level. Hyperglycemia is critical to the onset and complication of diabetes. Decreased insulin level as shown in this model demonstrates insulin independent diabetes (Figure 1) induced by streptozotocin which destroys the pancreatic β. cells. These conditions show important metabolic signals that result in severe pathological processes like oxidative stress, inflammatory responses, fibrosis, neuropathy, and eventual CVD. The decreased activities in Adenosine deaminase, xanthine oxidase and uric acid level as reported in this study is due to insulin resistance, hyperglycemia and renal dysfunction. These conditions resulted in purine dysmetabolism. Purine metabolism is associated with clearance of adenosine, a regulatory molecule known to inhibit the inflammatory responses elicited by tissue macrophages. Conversely, purine dysmetabolism, result in increased purine degradation products like adenosine, hypoxanthine, xanthine and uric acid via the xanthine oxidase pathway which raised inflammation, oxidative stress and tissue damage associated with diabetes mellitus. This is consistent with other studies which

have shown that adenosine resists oxidative damage and elevated ADA activity has been reported in T2DM (Kurtul *et al.,* 2005; Chen, Li and Zou, 2001) it has also been reported that adenosine improves antioxidant capacity *via* activation of adenosine receptors (A1 and A2A) in the kidney (Husain and Somani, 2005) and in endothelium (Zhang and Handy, 2005, Yasuda *et al.,* 2003).Uric acid acts as a strong antioxidant outside the cell and as a prooxidant inside the cell where it increased intracellular oxidative stress, mitochondrial injury, and ATP depletion by stimulating NADPH oxidase enzyme (Sanchez-Lozada *et al.,* 2012; Sautin *et al.,* 2007). XO is abundantly expressed in the liver and generates hydroxyperoxide when catalyzing substrates. Therefore, serum UA elevation is considered to accompany increased accumulation of oxidative stress generated by XO. Sodium acetate modulates purine metabolism through the Adenosine deaminase /Xanthine oxidase/Uric Acid pathway thereby suppressing oxidative stress and cell damage in diabetes.

Therefore, sodium acetate ameliorates purine dysmetabolism in DM by suppressing ADA and XO activities and UA production. This is in agreement with a report that XO inhibitor suppresses the development of diabetes associated nonalcoholic steatohepatitis in a rodent Model (Nakatsu *et al.,* 2015). The report of this study further confirms the role of UA in the development of metabolic syndrome. The restoration of purine metabolism by sodium acetate plasma through ADA/XO/UA activity (Fig. 2) improves inflammation, oxidative stress and tissue damages in DM (Michael and Olatunji, 2018).

Conclusion

In conclusion, the present study demonstrates that DM results in purine dysmetabolism thereby elevating the purine degradation products with increased plasma uric acid, ADA and XO activities and oxidative stress. However, sodium acetate regulated purine metabolism through ADA/XO/UA pathway, oxidative damage and augmentation of glycogen synthesis implicating the therapeutic importance of acetate against STZ-induced diabetes. Acetate would be a potential therapeutic intervention for diabetic patients particularly those with hyperuricemia.

Conflict of interest

The authors declare no conflict of interest.

Author's contributions

Dangana Elizabeth O and Ekpe Christian N design the study and contribute to the drafting of the manuscript, Negedu Muhammed N, Jonah Achile C F.O. Aliyu Fatima O, Akhigbe Faith O Benjamin Eleojo discussed and edited the manuscript.

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