Ascorbic Acid Pre-Treatment Prevents Diabetes In Alloxanised Fructose-Obese Wistar Rats

Dr Temitope Deborah Fabiyi-Edebor
Department of Physiology, College of Medicine and Health Sciences,
Afe Babalola University, Ado-Ekiti, Ekiti, Nigeria.
Email: fabiyitd@abuad.edu.ng; debbyteefab@gmail.com

***Abstract*— Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia and stress have been implicated in its etiology. Ascorbic acid, an aqueous phase antioxidant has been reported to improve glucose disposal in healthy and diabetic conditions. However, there seems to be a dearth of information on the possibility of ascorbic acid preventing diabetes. Thus, this study was carried out to investigate the effect of ascorbic acid pre-treatment on alloxan induced diabetes in fructose fed rats. Wistar rats (24) were randomly divided (n=6) into: control, fructose + alloxan (diabetic), fructose + ascorbic acid + alloxan, fructose + metformin + alloxan. Fructose(20%w/v) solution via gavage drinking lasted for 2 weeks. Then ascorbic acid (1g/kg) and metformin (500mg/kg) treatment was given for 2weeks before alloxan (150mg/kg) injection (i.p). After another 2weeks, blood was collected for fasting blood glucose and plasma insulin determination via spectrophotometry and ELISA respectively. Homeostatic model assessment (HOMA) was used to determine insulin resistance, insulin sensitivity and beta cell function. Data was analyzed using ANOVA at $p<0.05$. Insulin sensitivity, beta cell function, fasting insulin and glucose levels were increased ($p<0.001$) in the fructose-alloxan group. However, these remained similar to control ($p>0.05$) in ascorbic acid and metformin pre-treated groups. Insulin resistance was significantly ($p<0.01$) increased in fructose-alloxan but not in treated groups. Ascorbic acid pre-treatment inhibited the diabetogenic activities of alloxan in fructose fed rat via mechanisms related to its antioxidant properties. Thus, ascorbic acid may be used to prevent diabetes and ameliorate pre-diabetic state.**

***Keywords*—Anti-oxidant; oxidative stress; fructose; diabetes; Insulin resistance**

I. INTRODUCTION

Diabetes is a chronic non-communicable disease of global burden with over 8.5% of the world's adult population living with the disease [1]. Much attention is being given to the management of diabetes while its prevalence continues to rise at an epidermic rate, however, it is expedient to start looking closely into how to prevent the development of the disease so as to reduce the rate at which the epidemic is advancing globally. Diabetes is characterized by elevated blood glucose levels and metabolic derangement and has been termed a disease of multiple etiologies, resulting in insulin deficiency or resistance [1,2]. However, stress has been implicated in the etiology of type two diabetes [3].

Studies have shown an association between high consumption of sugar-sweetened beverages and increased risk of type 2 diabetes [4]. This is because fructose, a sweetener and an important component of sugar used to sweeten beverages, activates metabolic pathways that lead to triglyceride (fat) accumulation, dyslipidemia, impaired glucose homeostasis and insulin resistance [5]. Triglyceride accumulation increases the production of free radicals and adipokines which depletes endogenous antioxidants, oxidizes DNA, RNA, proteins and lipids in cells and activate pathways that further aggravates the metabolic derangement and insulin resistance leading to type 2 diabetes [6,7]. On another hand, oxidative stress has been said to precede obesity, causing insulin resistance in adipose and peripheral tissues and consequently resulting in diabetes [3,8,9]. Since oxidative stress is an important piece in the complex mechanism by which diabetes develops, the use of antioxidants may be beneficial in the prevention of diabetes. Clinical studies have shown that eating fruit and vegetable diet rich in antioxidants is associated with a lower incidence of diabetes [10]. This is understandable because antioxidants protect biological tissue from being damaged by free radicals. Studies have shown that antioxidants such as vitamin E (tocopherol), ascorbic acid (vitamin C) and lipoic acid to mention a few, are beneficial in ameliorating diabetic complications [3, 11]. Ascorbic acid is an aqueous phase antioxidant known to scavenge free radicals. It has been reported to improve whole body glucose disposal in both healthy and diabetic patients [12]. Furthermore, it has been shown to ameliorate some of the complications of diabetes [13,14]. However, there seems to be a dearth of information on the possibility of ascorbic acid preventing the onset of diabetes in high risk condition such as obesity. Thus, this study was carried out to investigate the effect of ascorbic acid pre-treatment on alloxan induced diabetes in fructose obese rats.

II. MATERIALS AND METHODOLOGY

A. *Animal Grouping*

Twenty-four adult male rats of wistar strain weighing between 150-200g were used for this study. They were maintained under standard housing conditions and had free access to normal rat chow and clean drinking water. In addition, they were cared for and humanely treated according to international guide for the care and use of laboratory animals [15]. The rats were randomly grouped (n=6) into control, fructose and alloxan, fructose + alloxan + ascorbic acid and fructose + metformin + alloxan. All, except the control group, received 20%w/v fructose solution freely via gavage drinking for two weeks. Thereafter, treatment with ascorbic acid (1mg/g/day) and metformin (0.5mg/g/day) via oral cannula took place for two weeks. Then diabetes was induced using 150mg/kg (i.p) injection of 5% alloxan while control group received normal saline vehicle intraperitoneally (i.p). The following measurements were taken from the rats two weeks after alloxan injection.

B. *Body weight*

Rat bod weight was measured using a weighing balance

C. *Fasting blood glucose*

Glucose level of blood from the tail vein of overnight fasted rats was determined spectrophotometrically.

D. *Fasting Plasma Inssulin*

Blood from the ocular sinus of fasting urethane-anesthetized rats was collected using a capillary tube. This was centrifuged to extract the serum from which insulin was determined via enzyme linked immunosorbent assay (ELISA).

E. *Metabolic measurements*

Insulin resistance, insulin sensitivity and beta cell function were determined non-invasively from fasting blood glucose and insulin variables using the homeostatic model assessment (HOMA) formulas:

1. Insulin Resistance (HOMA-IR)
   HOMA-IR = [FPI (IU/ml) × FBG (mg/dl)] / 22.5

2. Insulin sensitivity (HOMA-%S)
   HOMA-%S = (1 / [(insulin X glucose) X 22.5]) %

3. Beta cell function (HOMA- %β)
   HOMA- %β = [(20 x FPI)/ (FBG – 3.5)

The denominator of 22.5 is a normalizing factor and is the product of normal fasting plasma insulin (5 μU/mL) and normal fasting plasma glucose (4.5 mmol/L) typical of a "normal" healthy individual [16].

F. *Statistical Analysis:*

Results are presented as mean ± S.E.M. and data was analyzed with ANOVA at P<0.05

III RESULTS

The effects of pre-treatment of fructose fed wistar rats with 1mg/g ascorbic acid and metformin before diabetes induction on fasting blood glucose, insulin and body weight are shown in figures 1, 2 and 3. There was significant ($p<0.001$) decrease in body weight after ascorbic acid and metformin treatment but this was not significant after alloxan injection (figure 1). Alloxan significantly ($p< 0.001$) increased fasting blood glucose and insulin levels in the fructose-alloxan (F + A) group compared to control. However, these were not significantly ($p >0.05$) greater than the control in both the ascorbic acid and metformin pre-treated groups. Insulin resistance was significantly ($p<0.001$) after alloxanisation but then it was not significantly ($p>0.05$) increased in the pre-treated rats. Insulin sensitivity was not significantly ($p>0.05$) altered in ascorbic acid pre-treated group compared with metformin pre-treated group which had a significantly ($p<0.05$) decreased insulin sensitivity (figure 6). The beta cell function as shown in figure 5 was slightly but significantly ($p<0.05$) reduced with ascorbic acid pre-treatment compared with both control and metformin pre-treated rats.

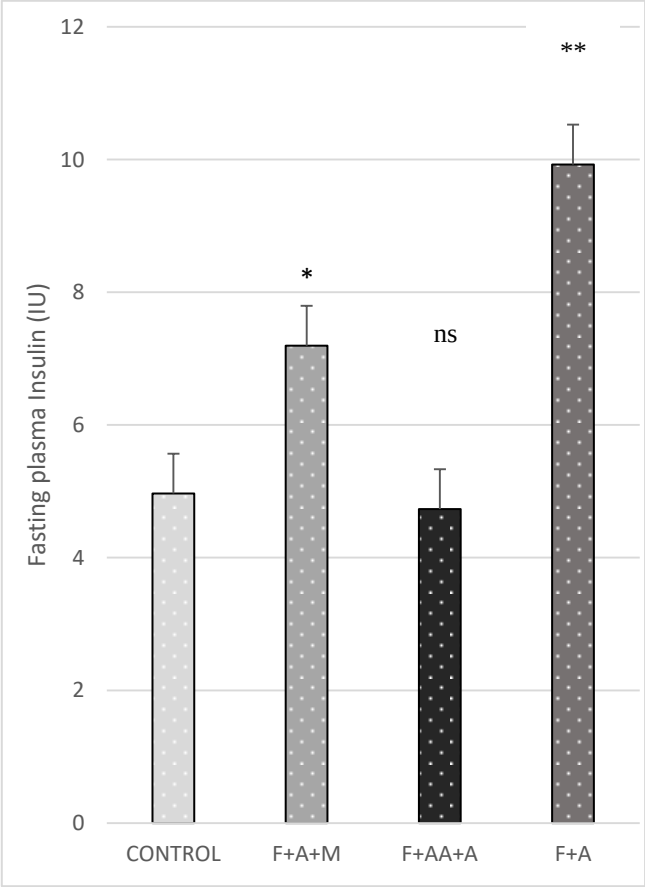

Figure 1: Different plasma insulin levels of control rats, pre-treated alloxanised rats (*F=Fructose, A=alloxan, AA= ascorbic acid and M=metformin). **p<0.001; ns- not significant relative to control.*

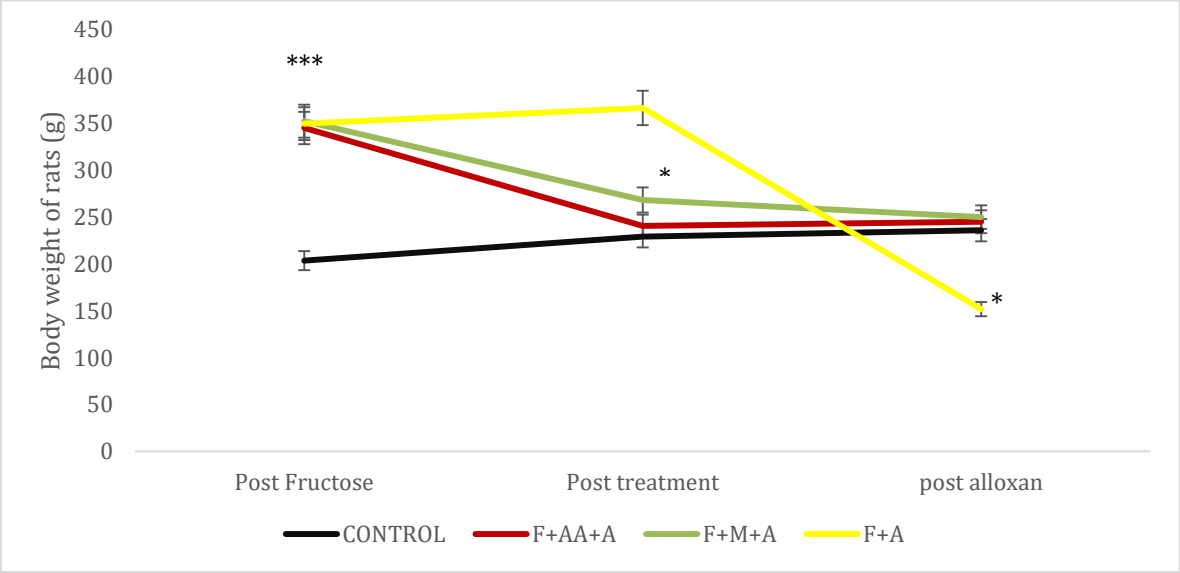

Figure 2: Body weight of ascorbic acid pre-treated rats before and after alloxan injection

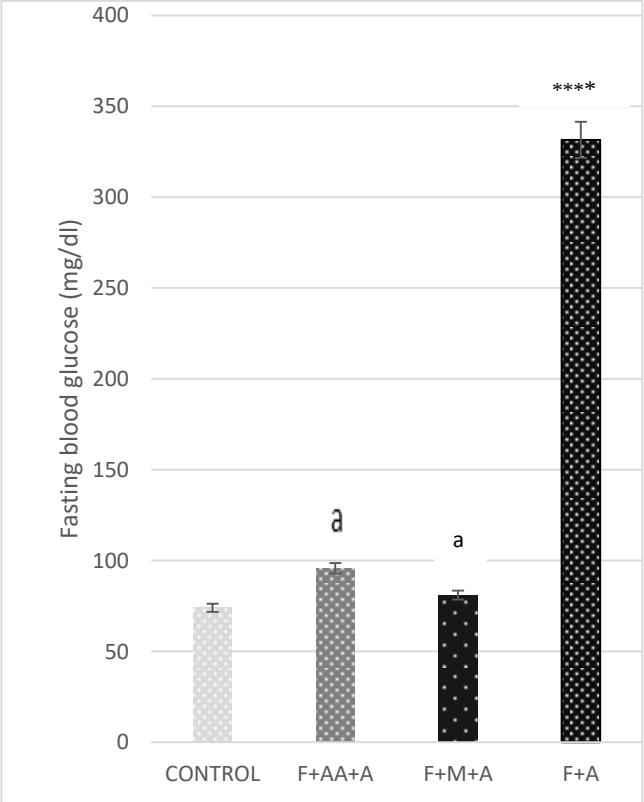

Figure 3: Fasting blood glucose levels of ascorbic acid pre-treated wistar rats after alloxanisation (*F=Fructose, A=alloxan, AA= ascorbic acid and M=metformin). **p<0.01, ***p<0.001, ****p<0.0001 different from control; ns- not significant relative to control*

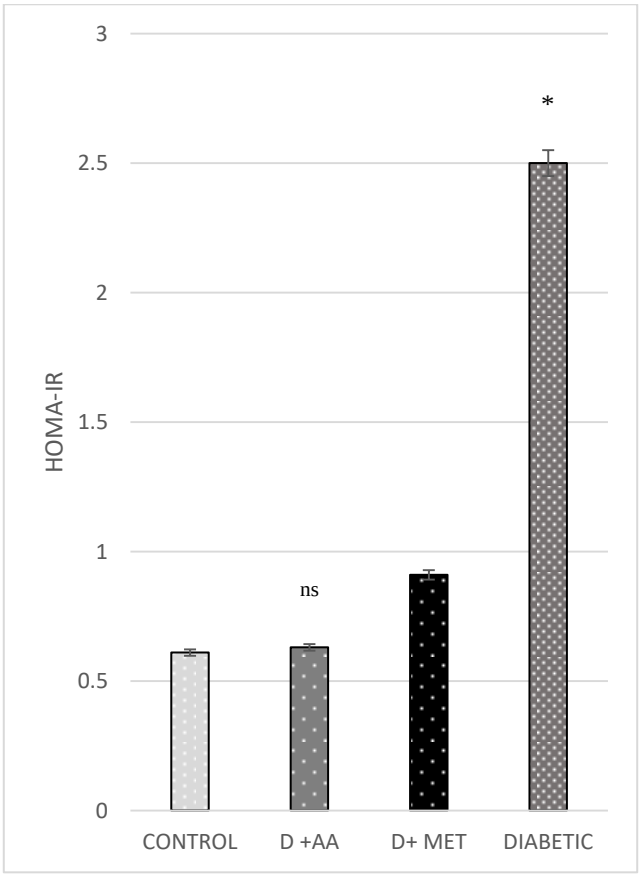

Figure 4: Ascorbic acid pre-treatment on Insulin resistance in fructose fed rats after alloxanisation. *D+AA (Fructose + ascorbic acid + alloxan), D+MET (Fructose + metformin + alloxan), DIABETIC (Fructose and alloxan).*

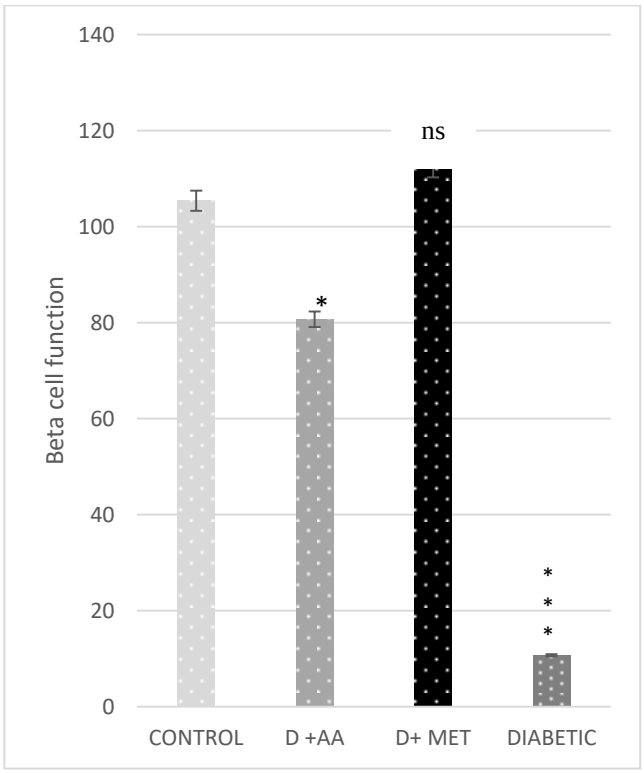

Figure 5: Ascorbic acid pre-treatment on beta cell function after alloxanisation. *D+AA (Fructose + ascorbic acid + alloxan), D+MET (Fructose + metformin + alloxan), DIABETIC (Fructose and alloxan).*

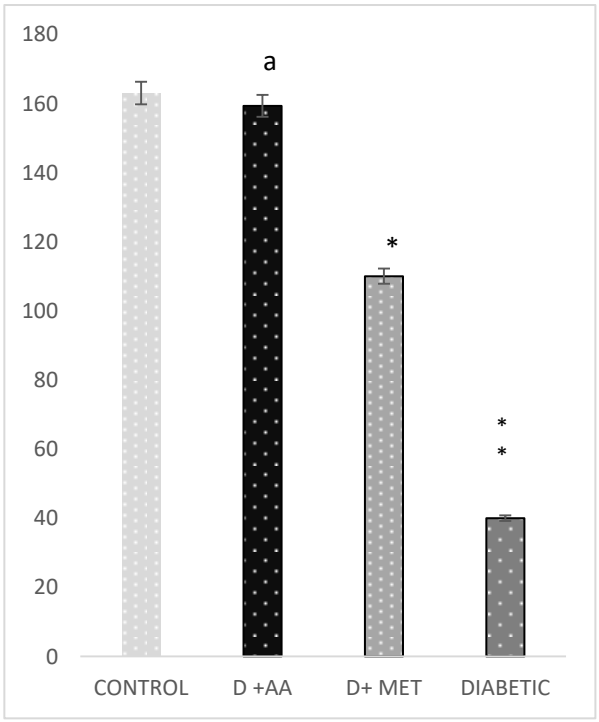

Figure 6: Ascorbic acid pre-treatment on insulin sensitivity post alloxanisation. *D+MET (Fructose and alloxan and metformin), D+ AA (Fructose and ascorbic acid and alloxan, DIABETIC (Fructose and alloxan).*

IV DISCUSSION

This study has demonstrated that ascorbic acid pre-treatment prevents the diabetogenic activities of alloxan in fructose fed wistar rats. Fructose has been observed to cause obesity which is a high risk for the development of diabetes or an intermediate stage in its progression [17]. Rats were alloxanized after fructose feeding to bring about the rapid development of diabetes that mimics human type 2 diabetes which is usually preceded by overweight or obesity. Alloxan is a beta cell toxic glucose analogue and a strong oxidizing agent capable of causing diabetes through its ability to selectively inhibit glucokinase in beta cells. It also, induces the formation of reactive oxygen species (ROS), such as super oxide radicals and other oxidative stress causing free radicals, resulting in the death of pancreatic beta cells [18] and consequently a decrease in beta cell function and insulin sensitivity followed by hyperinsulinemia as seen in this study. However, ascorbic acid treatment before alloxanization was able to prevent the activities of alloxan by protecting majority of the beta cells and preserving their functions as shown by the insignificant reduction in beta cell function and insulin sensitivity. This correlates with the reduction in superoxide formation and increased glutathione production evidently seen in ascorbic acid treated group in this study. Also, ascorbic acid inhibited lipid peroxidation as seen by the reduced MDA. Reducing MDA and superoxide generation and improving antioxidant capacity have been shown to improve beta cell function [19]. The increased production of endogenous antioxidant glutathione helped to prevent hyperinsulinemia in ascorbic acid treated rats as glutathione is known to protect beta cell glucokinase against alloxan inhibition thus preserved insulin regulating mechanisms [18,19]. Hyperinsulinemia is primarily responsible for insulin resistance which usually progresses into diabetes, thus preventing the mechanisms that bring about hyperinsulinemia would prevent diabetes. Ascorbic acid demonstrated the potential of preventing diabetes by preventing hyperinsulinemia and protecting beta cells in rats alloxanized after a high calorie fructose diet.

Fasting blood glucose was greatly elevated in untreated alloxanized rats however, there was normoglycemia in rats pre-treated with ascorbic acid before alloxanization. Elevated blood glucose, also known as hyperglycemia, is the major indicator of diabetes [20]. Thus, its absence is a pointer to the fact that there was normal glucose homeostasis, insulin sensitivity and beta cell function in the pre-treated rats, and that diabetes was not induced in them after alloxan injection. Anti-diabetogenic properties of ascorbic acid was compared to that of a common anti-diabetic drug called metformin. Metformin was found to prevent diabetes when administered prior to alloxanization. This was corroborated with the studies of Aroda and Ratner [21] who reported that metformin prevented the

development of diabetes in obese and high-risk individuals. Ascorbic acid and metformin also caused a reduction in body weight of fructose fed rats and their weight was stable after alloxanization unlike the untreated rats that experienced a drastic loss in body weight after alloxanization owing to the tissue wasting symptoms of diabetes. Thus, ascorbic acid may help in shedding excess weight and importantly, prevent the development of diabetes in high risk people such as the obese or overweight.

Conclusion

The use of ascorbic acid in the prevention of type 2 diabetes should not be overlooked as this study has demonstrated that ascorbic acid treatment in fructose-fed obese rats before alloxanization prevents the development of diabetes. Ascorbic acid may therefore prevent the progression of high risk or prediabetic states to diabetes. The mechanism by which ascorbic acid prevented the diabetogenic effect of alloxan might be by inhibiting its uptake by the beta cells, auto-oxidation, generation of free radicals and therefore protect beta cells from oxidative death. However, clinical trials may be required to ascertain this finding in humans.

Conflict of interest

The author declares no conflict of interest

Ethical approval

Experiments were carried out as approved by ABUAD animal research ethical committee.

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