

Liver Enzymes and Hepatic Histomorphological Assessment in Alloxan Monohydrate Induced Hyperglycaemic Male Wistar Rats treated with Glibenclamide and Theophylline

Abstract

Aim: Increase in blood glucose level has been associated with beta cells destruction which is enhanced by lipid peroxidation and hepatocellular damage. The present study was designed to investigate the effect of oral administration of theophylline on Liver enzymes (ALT, AST and ALP) and Liver histology in alloxan-induced diabetic male wistar rats.

Study design: Thirty healthy male wistar rats weighing between 160-180g were grouped into five of six animals each (n=6) and treated for a period of fourteen days (14) after induction of hyperglycaemia using alloxan monohydrate as follows; Group 1: (Normoglycaemic) Group 2: Diabetic control, Group 3: Glibenclamide, 5mg/kg, Groups 4 and 5; theophylline 5mg/kg and 10mg/kg respectively. At the end of the 2 weeks study period, rats were anesthetized using chloroform inhalation in a closed chamber. Blood samples were taken from all groups for evaluation of serum ALT, AST and ALP levels. Liver tissues were also harvested from experimental groups and liver histology carried out.

Results: Although there was no statistically significant difference observed in serum ALP and ALT levels compared to the diabetic control, however there was a significant decrease ($P < 0.05$) in serum level of AST in the theophylline treated groups compared to the diabetic control. There was mild decrease in fatty infiltrations and hepatocellular degeneration observed in the liver histology from the theophylline treated groups.

Conclusion: Theophylline at 5 and 10 (mg/kg) per os decreased serum liver enzymes with mild regeneration of hepatocytes in alloxan induced hyperglycaemic male Wistar rats

Keywords: Theophylline, Liver enzymes, Hyperglycaemia and Alloxan

1.0 INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder characterized by abnormally elevated level of blood glucose because of deficiency in insulin secretion by the (beta) β cells of pancreas and or resistance toward the action of anti-diabetic hormone insulin associated with disturbances in the carbohydrates, lipids, and proteins metabolism which lead to macro and microvascular dysfunction and long term health complications [1]. It is characterized by abnormally elevated levels of blood glucose due to complete or relative insufficiency in insulin secretion and /or insulin action together with chronic hyperglycaemia (high fasting glucose level above 126 mg/dl) as well as disturbances in carbohydrate, fat and

protein metabolism [2]. Theophylline is a member of the xanthine family, also known as 1, 3-dimethylxanthine and has been used in the treatment of asthma, chronic obstructive pulmonary disease and apnoea [3]. Theophylline is metabolized in the liver extensively up to about 70%. It undergoes N-demethylation via the Cytochrome P450 1A2 and is excreted in the urine up to about 10% [4]. The mechanism of action of alloxan monohydrate in β cells of the pancreas is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells [5]. The liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis [6].

1.1 EXPERIMENTAL SITE

The study was carried out in the Physiology Laboratory of the Department of Human Physiology, Faculty of Medical Sciences, College of Health Sciences, Ahmadu Bello University. Zaria is located between latitudes 11° and 3° N, and between 7° and 42° E, at an altitude of 670 m above the sea level and 664 km away from the sea, in the Northern Guinea Savanna zone [7] 

1.2 CHEMICALS AND EQUIPMENTS

Alloxan monohydrate, formalin, normal saline were of analytical grade and purchased from Sigma Aldrich chemical Company (St. Louis U.S.A.). Glibenclamide tablets, 5mg (Clamide, Hovid Pharmaceuticals Ltd, Malaysia) NAFDAC Reg. no. 04-4015) and Theophylline, 100 mg (Theolair, Mancare Pharmaceuticals Ltd, India  NAFDAC Reg. no. A4-9071) were purchased from the Pharmacy section of Ahmadu Bello Teaching Hospital, Zaria, Kaduna

state. A Digital Glucometer (Accu-Chek Advantage, Roche Diagnostic), Germany, Weighing Balance (GF 2000), Dissecting set, Petri-dish, , plain bottles, EDTA tubes, cotton wool, Denley BS400 Centrifuge (England) syringes and needles were used.

1.3 EXPERIMENTAL DESIGN

Thirty (30) adult male Wistar rats weighing between 160-180 g were purchased from the Animal house of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria. The rats were housed in the Animal house of the Department of Human Physiology under laboratory conditions with access to food and water *ad libitum*.

1.3.1 Induction and Confirmation of Diabetes mellitus

Diabetes was induced by single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline solution into the Wistar rats of groups III, IV and V after 16-18hrs fasting of the Wistar rats [8]. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin secretion; the induced Wistar rats was treated with 20% glucose solution orally within 6 hrs. The Wistar rats were kept for the next 24 hrs on 5% glucose solution in their cages to prevent hypoglycaemia [9]. After 72 hours of alloxan treatment, blood was collected from tail vein of each rat and assessment of blood glucose level was carried out using a digital Glucometer. Rats having fasting blood glucose level 180 mg/dl and above were considered diabetic [10]

1.3.2 Animal grouping and treatment

Groups	No. of Rats	Treatment (dosage/kg body weight)	Duration
I.	6	Distilled water 1ml/kg daily (Normoglycaemic)	14 days
II.	6	Diabetic + Distilled water 1ml/kg daily	14days
III.	6	Diabetic + 5 mg/kg (Glibenclamide)	14 days
IV.	6	Diabetic + 5 mg/kg theophylline	14 days

V.	6	Diabetic + 10 mg/kg theophylline	14 days
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80 **1.4 STATISTICAL ANALYSIS**

81 Data obtained from the study were expressed as mean $\pm$ SEM. Statistical analysis was
 82 carried out using version 20 of SPSS with the aid of one way analysis of variance (ANOVA)
 83 and Tukey's post-hoc test. Values with ($P < .05$) were considered significant

84 **2.0 RESULT**

85 **2.1 Effect of fourteen (14) days Oral administration of Theophylline and Glibenclamide** 86 **on serum alkaline phosphatase (ALP) level in Alloxan induced Male Diabetic Wistar** 87 **rats**

88 In Fig 1 below the serum level of ALP was not significant in the theophylline (5 mg/kg and 10
 89 mg/kg) treated groups; ($119.75 \pm 3.29 \mu\text{L}$ and $115.75 \pm 2.61 \mu\text{L}$) respectively, when
 90 compared to the diabetic control group. There was however a significant decrease in the
 91 Glibenclamide treated group; $75.60 \pm 1.79 \mu\text{L}$ when compared to the DC ($P < .05$)

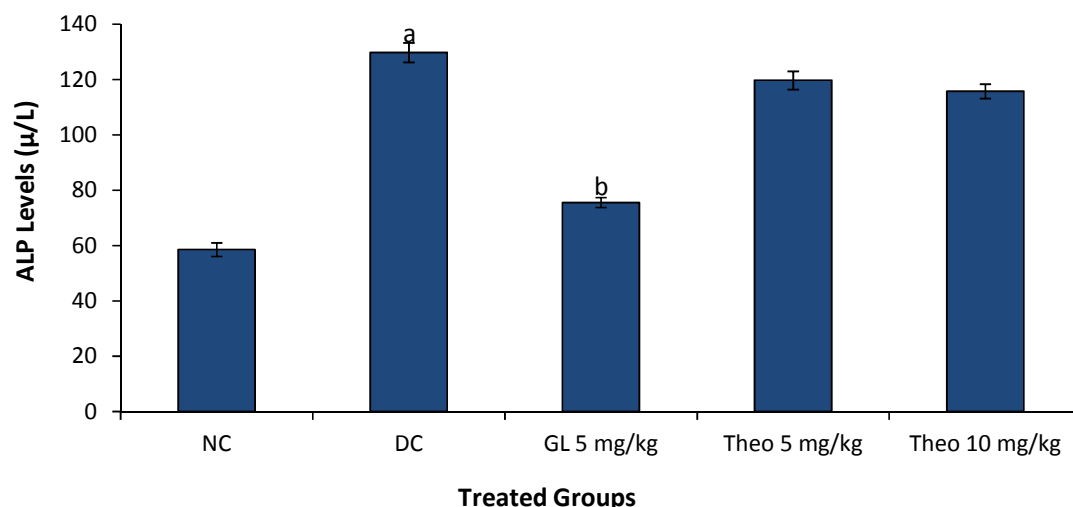


Figure 1: showing serum Alkaline phosphatase level following treatment with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant ($P < .05$) compared to normal control (NC), b= statistically significant ($P < .05$) compared to diabetic control DC.

2.2 Effect of fourteen (14) days Oral administration of Theophylline and Glibenclamide on serum alanine amino transferase (ALT) level in Alloxan induced Male Diabetic Wistar rats

In Fig 2 below the serum level of ALT was not statistically significant in the theophylline (5 mg/kg and 10 mg/kg) treated groups; ($49.50 \pm 1.75 \mu/L$ and $48.50 \pm 1.44 \mu/L$) respectively, when compared to the diabetic control group. There was however a significant decrease in the Glibenclamide treated group; $39.80 \pm 2.06 \mu/L$ when compared to the DC ($P < .05$).

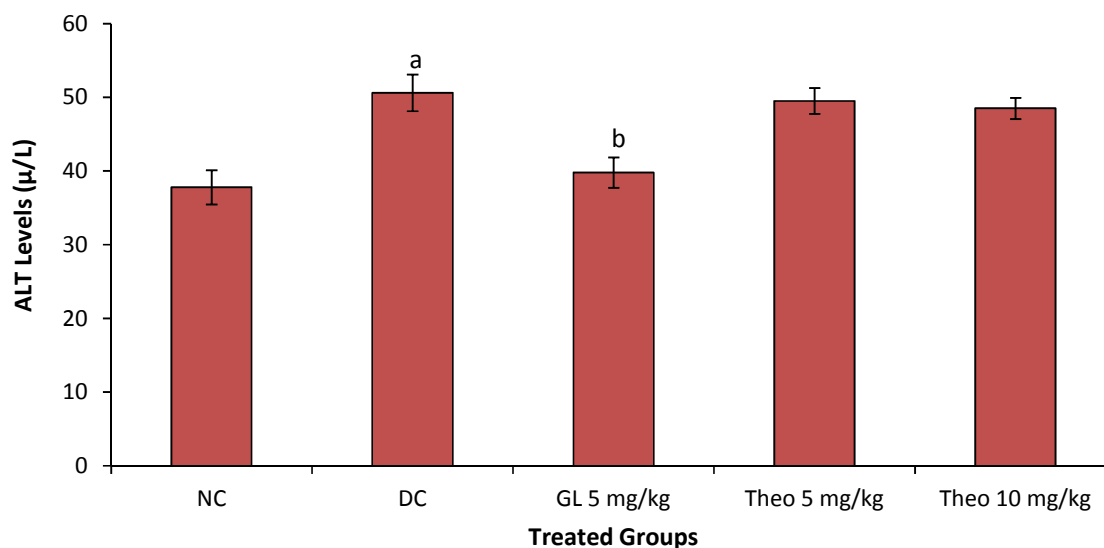


Figure 2: Showing Alanine amino transferase following treatment with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant ($P < .05$) compared to normal control (NC), b= statistically significant ($P < .05$) compared to diabetic control DC.

2.3 Effect of fourteen (14) days Oral administration of Theophylline and Glibenclamide on serum aspartate amino transferase (AST) level in Alloxan induced Male Diabetic Wistar rats.

In Fig.3 below, the serum level of AST was decreased non-significantly in the theophylline (5 mg/kg and 10 mg/kg) treated groups; ($47.60 \pm 2.07 \mu/L$ and $46.20 \pm 0.86 \mu/L$) respectively, when compared to diabetic control group ($P < .05$).

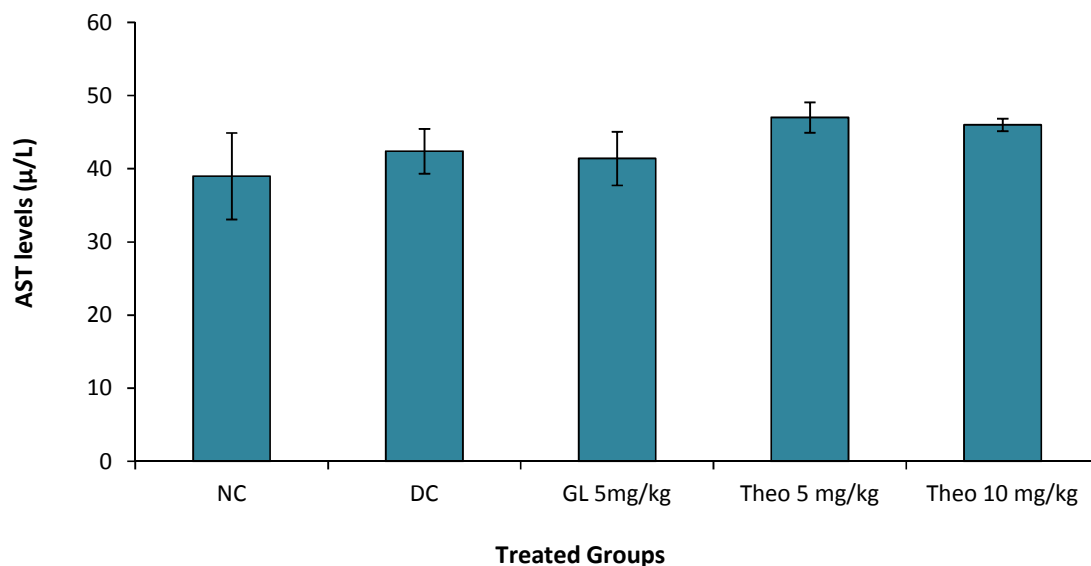


Figure 3: Showing Alanine amino transferase following treatment with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide.

2.4 Effect of fourteen (14) days oral evaluation of theophylline and glibenclamide on histomorphology of the liver

Photomicrograph sections of the liver after 14 days of oral administration of Control (normal saline) (1 ml/kg), Diabetic control, Glibenclamide (5 mg/kg), theophylline (5 mg/kg) and (10mg/kg) in plate I, II, III, IV and V respectively. The theophylline treated groups showed mild decrease in fatty infiltration.

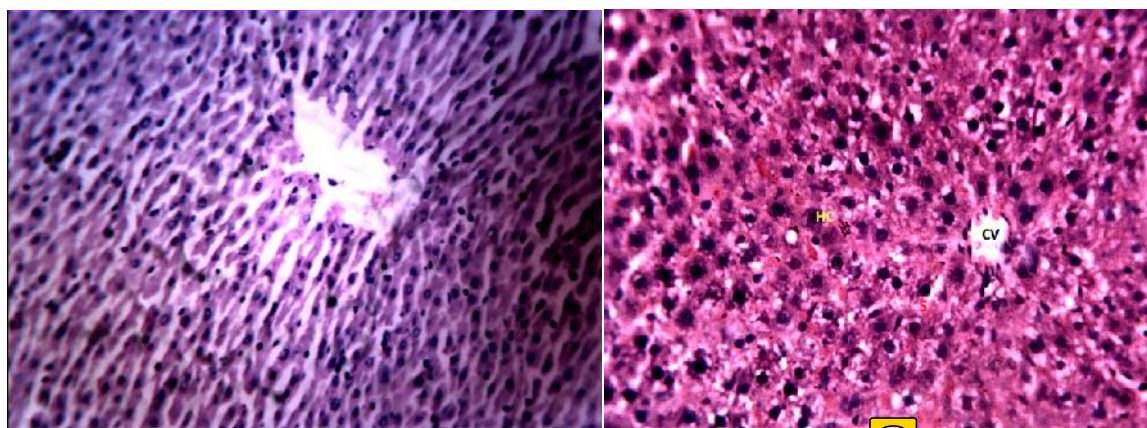


PLATE I: Diabetic control

Plate II: Glibenclamide treated (5 mg/kg)

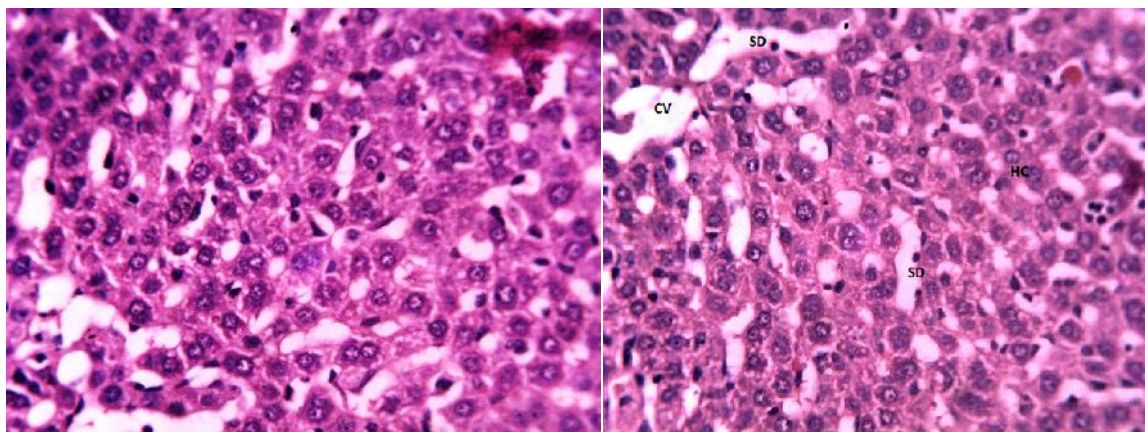


Plate III: Theophylline treated (5 mg/kg)

Pate IV: Theophylline treated (10 mg/kg)

H and E stain, x250 CV = central vein, HC = hepatocytes, S= Sinusoidal dilatation

3.0 DISCUSSION

The increase in serum liver enzymes ALP, ALT and AST in the DC compared to the NC as shown in figures 1, 2 and 3 above would be from the toxic effect of alloxan monohydrate. Increase serum liver enzymes level is an indication of hepatocellular destruction typical to diabetic complications. The decrease observed in the glibenclamide treated group could have been due to its effect on hyperglycaemia which indirectly reduces hepatocellular destruction by mitigating lipid peroxidation. This present study support the findings of [11], who reported that increase in the liver ALT and AST, reveal impaired liver function. The decrease in the liver enzymes in theophylline treated group could have been because of theophylline's coffee component which has been reported to decrease and elevated levels of liver enzymes. [12] proposed that decaffeinated coffee intake lower liver enzyme levels promoting liver health. The result of theophylline oral administration observed on liver enzymes ALP and ALT suggests a possible hepatoprotective effect which is in concert to the result of the liver histomorphology. Histopathology evaluation of the liver tissues showed mild fatty changes in centrolobular portion of the liver in the diabetic rats. The Fatty infiltrations however were seen to decrease as shown in the photomicrograph of the theophylline treated tissues. This could have been mediated through decrease in the activity of the glycogen synthase enzymes, consequently reducing or inhibiting the faulty deposition of glycogen in the diabetic rats. In plate III and IV above, there was observed a decrease in

desquamation of the hepatocytes along side restoration of a normal histoarchitecture relative to the diabetic control.

3.1 CONCLUSION

Theophylline at 5 and 10 (mg/kg) per os decreased serum liver enzymes with mild regeneration of hepatocytes in alloxan induced hyperglycaemic male Wistar rats.

3.2 REFERENCES

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