

Lipid peroxidation and Antioxidant Enzymes Assessment in Alloxan Monohydrate Induced Hyperglycaemic Male Wistar Rats following Oral Administration of Theophylline and Glibenclamide

ABSTRACT

Aim: Lipids are reported as one of the primary targets of reactive oxygen species (ROS). The main sources of oxidative stress in diabetes mellitus comprise various enzymatic pathways, non-enzymatic pathways and mitochondrial pathways. This study was designed to evaluate the effect of theophylline and glibenclamide treatment on lipid peroxidation (serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase, glutathione peroxidase and Catalase) in alloxan induced hyperglycaemic 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.

Methodology: Group 1: (Normoglycaemic) Group 2: Diabetic control (DC), Group 3: Glibenclamide, 5mg/kg, Groups 4 and 5; theophylline 5mg/kg and 10mg/kg respectively. At the end of the fourteen (14) days, rats were anesthetized using chloroform inhalation in a closed chamber. Blood samples were taken from all treated groups for evaluation of serum MDA, SOD, GPx and CAT level.

Results: The result on serum MDA concentration was significantly decreased ($P < 0.05$) in glibenclamide treated group compared to diabetic control; 1.14 ± 0.03 vs 1.32 ± 0.06 . Although a decrease was observed in the theophylline treated groups, the difference was however not statistically significant compared to diabetic control. There was also significant increase ($P < 0.05$) in serum SOD and CAT level in the glibenclamide and theophylline treated group (5 mg/kg) compared to DC; 2.02 ± 0.04 and 1.92 ± 0.24 vs 0.92 ± 0.05 respectively for serum SOD and 53.20 ± 0.58 and 52.80 ± 1.07 vs 46.00 ± 0.84 respectively for CAT. However, serum GPx increased significantly ($P < 0.05$) only in the theophylline treated groups compared to DC.

Conclusion: In conclusion, Theophylline and Glibenclamide decreases lipid peroxidation while increasing serum antioxidant levels in alloxan induced hyperglycaemic male Wistar rats after 14 days oral administration.

Keywords: Lipid peroxidation, Antioxidant enzymes, Theophylline, Glibenclamide, Hyperglycaemia.

1.0 Introduction

Diabetes mellitus is the commonest endocrine-metabolic disorder characterized by chronic hyperglycemia giving to the risk of microvascular (Retinopathy, nephropathy and

neuropathy) and macrovascular (ischemic heart disease, stroke and peripheral vascular disease) damage, with associated reduced life expectancy and diminished quality of life [1]. Diabetes mellitus remains a burdensome and public health problem with increasing global incidence [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 mostly found in cocoa and coffee possesses antioxidant activity which have been attributed to their enhancing effect on cellular cyclic nucleotides contributing to protection against hyperglycaemia and oxidative stress that leads to diabetic complications [4]. Theophylline is also a phosphodiesterase enzyme inhibitor with recent evidences indicating it has effect on reverting induction of oxidative [5, 6, 7]. Glibenclamide is one of the most frequently prescribed oral hypoglycemic agents [8]. It stimulates insulin secretion and also reduces hepatic glucose production resulting in reduced blood glucose [9]. However, the use of glibenclamide is limited due to prolonged hypoglycemia, high secondary failure rate and other adverse events [10, 11]. Oxidative stress plays a pivotal role in cellular injury from hyperglycaemia. High glucose level can stimulate free radical production. Weak defence system of the body becomes unable to counteract the ROS generation and as a result, condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress [12, 13]

1.1 Material and Methods

1.1.1 Experimental site

This 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 [14]

1.1.2 Chemicals and Reagents

Chemicals which were used include; alloxan monohydrate, formalin, normal saline, they 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, 100mg (Theolair, Mancare Pharmaceuticals Ltd, India NAFDAC Reg. no. A4-9071) were purchased from the Pharmacy section of Ahmadu Bello Teaching Hospital, Zaria, Kaduna state Nigeria.

1.1.3 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*.

1.1.4 Induction and Confirmation of Hyperglycaemia

Diabetes (Hyperglycaemia) 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 [15]. 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 [16]. 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 [17].

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92 **1.1.5 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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94 **1.2 Statistical analysis**

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

98 **2.0 Results**

99 **2.1 Effect of fourteen (14) days Oral administration of Theophylline and Glibenclamide**
 100 **on serum Malondialdehyde (MDA) level in Alloxan induced Hyperglycaemic Male**
 101 **Wistar rats.**

102 In Table 2.1 below, the serum levels of malondialdehyde (MDA), in the theophylline treated
 103 groups; $1.30 \pm 0.10 \mu\text{mol/L}$ and $1.30 \pm 0.10 \mu\text{mol/L}$ (5 mg/kg and 10 mg/kg respectively)
 104 were not statistically significant when compared to the diabetic control group. However, there
 105 was observed a significant decrease in the glibenclamide treated groups

106 Table 2.1: Serum malondialdehyde concentrations in Alloxan induced Hyperglycaemic Male
 107 Wistar rats.

Treated Groups	MDA($\mu\text{mol/L}$)
Normal control (NC)	1.08 ± 0.04

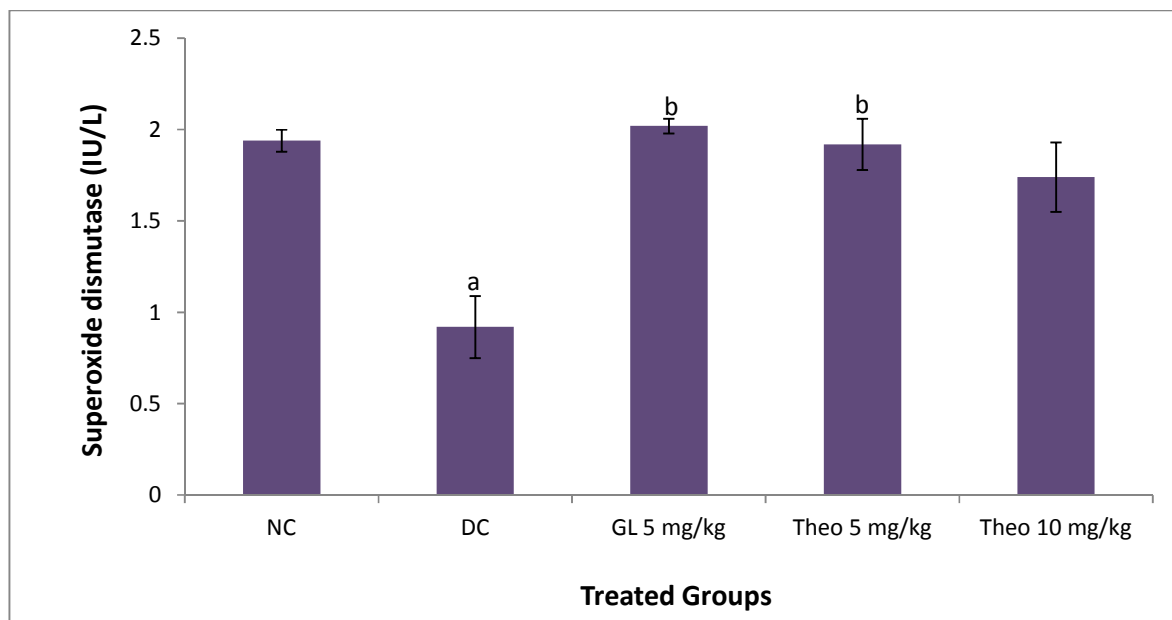
Diabetic Control (DC)	1.32 ± 0.06
Glibenclamide (5 mg/kg)	1.14 ± 0.03 ^b
Theophylline (5 mg/kg)	1.30 ± 0.10
Theophylline (10 mg/kg)	1.30 ± 0.10

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109 **2.2 Effect of fourteen (14) days Oral administration of Theophylline on serum**
110 **Superoxide dismutase (SOD) level in Alloxan induced Hyperglycaemic Male Wistar**
111 **rats.**

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113 In Fig 2.1 below, the serum level of superoxide dismutase (SOD), in the theophylline (5
114 mg/kg) treated group; 1.92 ± 0.24 IU/L was significantly increased when compared to the
115 diabetic control group ($P < 0.05$), there was an insignificant increase in the 10 mg/kg
116 theophylline treated group when compared to the diabetic control. In the glibenclamide
117 treated group; 2.02 ± 0.04 IU/L recorded a statistical significant increase when compared
118 with the diabetic control ($P < 0.05$)



119

120 Figure 2.1: showing serum SOD level following treatment with glibenclamide and
121 theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL=
122 glibenclamide. Superscripts a= statistically significant ($P < .05$) compared to normal control

(NC), a= statistically significant compared to NC, b= statistically significant ($P < .05$) compared to diabetic control DC.

2.3 Effect of fourteen (14) days Oral administration of Theophylline on serum Catalase (CAT) level in Alloxan induced Hyperglycaemic Male Wistar rats.

In Fig 2.2 showing the serum level of catalase below was significantly increased in the Glibenclamide (5 mg/kg) and theophylline (5 mg/kg) treated groups; (53.20 ± 0.58 IU/L and 52.80 ± 1.07 IU/L) respectively, when compared to the diabetic control group ($P < 0.05$).

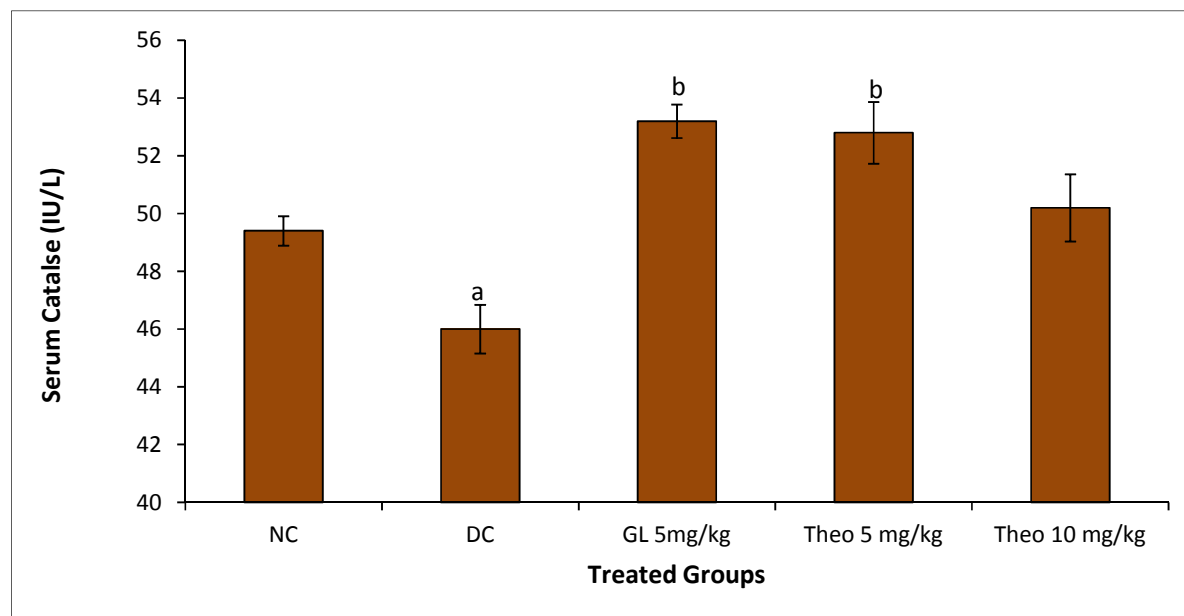
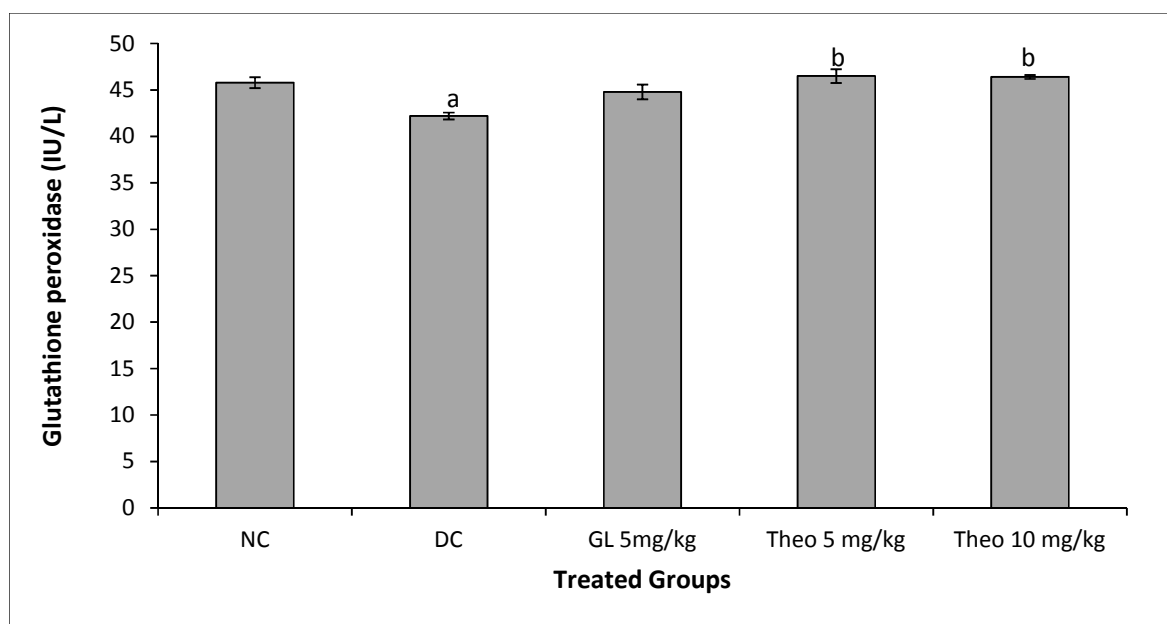


Figure 2.2: showing serum SOD level following treatment with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant ($P < .05$) compared to normal control (NC), a= statistically significant compared to NC, b= statistically significant ($P < .05$) compared to diabetic control DC.

2.4 Effect of fourteen (14) days Oral administration of Theophylline on serum Glutathione peroxidase (GPx) level in Alloxan induced Male Diabetic Wistar rats.

In Fig 2.3 below, the serum level of GPx was significantly increased in the theophylline (5 mg/kg and 10 mg/kg) treated groups; (46.50 ± 1.34 IU/L and 46.50 ± 1.21 IU/L) respectively, when compared to the diabetic control group ($P < 0.05$).

145



146

147 Figure 2.3: showing serum SOD level following treatment with glibenclamide and
 148 theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL=
 149 glibenclamide. Superscripts a= statistically significant ($P < .05$) compared to normal control
 150 (NC), a= statistically significant compared to NC, b= statistically significant ($P < .05$)
 151 compared to diabetic control DC

152 3.0 Discussion

153 A search for ways to reduce oxidative damage has become the focus of interest for patients
 154 with diabetes. Not only have increases level of reactive oxygen species and reactive
 155 nitrogen species been associated with oxidative damage but also reduced antioxidant
 156 defences [18]. The increase in serum MDA concentration observed in this current study, in
 157 the diabetic control would be from the pro peroxidative activity of alloxan monohydrate
 158 administration. This action of alloxan is mediated by reactive oxygen species. Alloxan and
 159 the product of its reduction, dialuric acid, establish a redox cycle with the formation of
 160 superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter
 161 highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive
 162 oxygen species with a simultaneous massive increase in cytosolic calcium concentration
 163 causes rapid destruction of β cells [19]. The mechanism by which hyperglycaemia leads to
 164 lipid peroxidation is the reduction of molecular oxygen and production of oxygen free radicals

and alpha-ketoaldehydes which finally breaks down lipid peroxides [20]. The action of theophylline on serum MDA concentration could be attributed to its antioxidant capacity due to its affiliation with coffee. Theophylline mostly found in cocoa and coffee possesses antioxidant activities which have been attributed to their enhancing effect on cellular cyclic nucleotides contributing to protection against hyperglycaemia and oxidative stress in diabetic complications. The activity of theophylline in this study could also have been through direct mopping out of the reactive oxygen or nitrogen species, serving as an impediment to progressive lipid peroxidation. This antioxidant activity is also represented in the result of serum SOD level, which was increased following theophylline administration. This activity could have been due to theophylline pro-activity on endogenous SOD synthesis. Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the dismutase of superoxide anion into hydrogen peroxide and molecular oxygen [21, 22]. In endothelial cells, it has been reported that hyperglycaemia increases hydrogen peroxide production causing a deficiency in catalase enzyme [23]. This is in concert with the decrease in serum CAT level observed in the diabetic control group. The depletion could also have been from consistent usage in mopping out the ROS and RNS. However, an increase was observed in the glibenclamide and theophylline treated groups which suggests that these substances possess antioxidant activities. This result is also in concert to the result of serum glutathione peroxidase (GPx) which shows increase in the serum level of GPx following theophylline and glibenclamide treatment. Glutathione disulfide is recycled back to glutathione by glutathione reductase; using the cofactor NADPH generated by glucose 6- phosphate dehydrogenase [24].

3.1 Conclusion

Theophylline and glibenclamide possesses a potent antioxidant activity and also decreases lipid peroxidation by decreasing serum malondialdehyde concentration in alloxan monohydrate induced hyperglycaemic male Wistar rats treated for fourteen days.

3.2 References

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