

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: This is an experimental study. Thirty healthy male wistar rats weighing between 160-180g were grouped into five of six animals each (n=6) following the induction hyperglycaemia using alloxan monohydrate and treated for a period of fourteen days (14) 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 experiment, blood samples were taken from all treated groups and sera obtained. Serum levels of ALT, AST and ALP were measured. Liver tissues were also harvested from experimental groups and histological assessment carried out.

Results: There were no statistically significant differences observed in serum ALP, ALT and AST levels in the theophylline treated groups compared to the diabetic control. The levels of ALP and ALT were however significantly higher ($P < 0.05$) in the theophylline treated groups than that observed in the glibenclamide treated group. Serum levels of ALP and ALT were significantly lowered ($P < 0.05$) in the glibenclamide treated group compared to both the diabetic control and the theophylline treated groups. Although less fatty infiltrations and hepatocellular degeneration were observed in the liver tissues of theophylline and glibenclamide treated groups compared to diabetic control, the effect was more in the glibenclamide treated group compared to the theophylline treated groups.

Conclusion: In this study, glibenclamide recorded less hepatotoxic activity by significantly lowering serum liver enzymes; ALP and ALT in contrast to theophylline which showed no significant differences in levels of serum enzymes in alloxan induced hyperglycaemic rats.

Keywords: Theophylline, Liver enzymes, Hyperglycaemia and Alloxan

1.0 INTRODUCTION

Diabetes mellitus as a persistent metabolic disorder is characterized by abnormally elevated level of blood glucose because of the deficiency in insulin secretion by the ~~(beta)~~ beta cells (β -cells) of pancreas or resistance toward the action of anti-diabetic hormone insulin associated with disturbance in the carbohydrates, lipids, and proteins metabolism, leading to macro and microvascular dysfunction [1]. It is also characterized with chronic hyperglycaemia (high fasting glucose level above 126 mg/dl [2]. Theophylline is a member of

37 the xanthine family, known as 1, 3-dimethylxanthine, and has been used to treat asthma,
38 chronic obstructive pulmonary disease and apnoea [3].

39 Theophylline is metabolized in the liver extensively up to about 70%. It undergoes N-
40 demethylation via the Cytochrome P450 1A2 and is excreted in the urine up to about 10%
41 [4]. The mechanism of action of alloxan monohydrate in β cells of the pancreas is mediated
42 by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a
43 redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to
44 hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton
45 reaction. The action of reactive oxygen species with a simultaneous massive increase in
46 cytosolic calcium concentration causes rapid destruction of β cells [5]. The liver plays a
47 major role in blood glucose homeostasis by maintaining a balance between the uptake and
48 storage of glucose via glycogenesis and the release of glucose via glycogenolysis and
49 gluconeogenesis [6]. Therefore, this study was aimed at evaluating the effects of
50 theophylline treatment on some hepatic enzymes and histology in alloxan monohydrate
51 induced diabetic Male Wistar rats.

52 1.1 EXPERIMENTAL SITE

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

58 1.2 CHEMICALS AND EQUIPMENTS

59 Alloxan monohydrate, formalin, normal saline were of analytical grade and purchased from
60 Sigma Aldrich ~~chemical-Chemical company-Company~~ (St. Louis U.S.A.). Glibenclamide
61 tablets, 5mg (Clamide, Hovid Pharmaceuticals Ltd, Malaysia, NAFDAC Reg. no: 04-4015)

62 and Theophylline, 100 mg (Theolair, Mancare Pharmaceuticals Ltd, India, NAFDAC Reg. no:
63 A4-9071) were purchased from the Pharmacy section of Ahmadu Bello Teaching Hospital,
64 Zaria, Kaduna state. Dissecting set, Petri-dish, plain bottles, cotton wool, Denley BS400
65 Centrifuge (England) syringes and needles were used.

66 **1.3 EXPERIMENTAL DESIGN**

67 Thirty (30) adult male Wistar rats weighing between 160-180 g were purchased from the
68 Animal house of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello
69 University Zaria. The rats were housed in the Animal house of the Department of Human
70 Physiology under laboratory conditions with access to food and water *ad libitum*. Ethical
71 approval was obtained from the Ahmadu Bello University ethical committee on animal use
72 and care, and the research was carried out according to the guidelines of Ahmadu Bello
73 University animal use and care policy.

74 **1.4 INDUCTION AND CONFIRMATION OF DIABETES MELLITUS**

75 Diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 150
76 mg/kg body weight dissolved in 0.9% cold normal saline solution into the Wistar rats of
77 groups 2, 3, 4 and 5 after 16-18hrs fasting of the Wistar rats [8]. Since alloxan is capable of
78 producing fatal hypoglycaemia as a result of massive pancreatic insulin secretion; the
79 induced Wistar rats were served with 20% glucose solution orally within 6 hrs post alloxan
80 monohydrate injections. The Wistar rats were kept for the next 24 hrs on 5% glucose
81 solution in their cages to prevent hypoglycaemia [9]. After 72 hours of alloxan treatment,
82 blood was collected from the tails of treated animals using a sterile blood lancet and
83 assessment of blood glucose level was carried out using a digital Glucometer. Animals with
84 fasting blood glucose level of 180 mg/dl and above were considered diabetic and used for
85 the study [10].

86

87

1.4 ANIMAL GROUPINGS AND TREATMENTS

Table 1: Groupings and respective treatments

Groups	No. of Rats (n)	Treatment (mg/kg b.w)
1	6	Distilled water (1 ml/kg) Normoglycaemic
2	6	Diabetic +Distilled water (1 ml/kg)
3	6	Diabetic +Glibenclamide (5 mg/kg)
4	6	Diabetic +Theophylline (5 mg/kg)
5	6	Diabetic +Theophylline (10 mg/kg)

1.5 Evaluation of Liver Enzyme

1.5.1 Determination of serum alkaline phosphatase (ALP)

Serum alkaline phosphatase was estimated according to the method [11]. This was carried out by adding 0.5 mL of the reagent to 0.05 mL of the samples then mixed and incubated at 37 °C for 10 minutes. The change in the absorbance of the sample was measured per minute ~~spectrophotometrically~~- spectrophotometrically at a wavelength of 590 nm.

1.5.2 Determination of serum aspartate aminotransferase (AST)

Serum aspartate aminotransferase was estimated by the method described by [12] where 1000 µL of the reagent was added to 100 µL of the sera and then mixed and incubated at 37 °C for 1 minute. The change in the absorbance of the samples was measured per minute ~~spectrophotometrically~~- spectrophotometrically at a wavelength of 590 nm.

1.5.3 Determination of serum alanine aminotransferase (ALT)

This was estimated by the method described by [12]. 1000 µL of the reagent was added to 100 µL of the sera and then mixed and incubated at 37 °C for 1 minute. The change in the absorbance of the samples was measured per minute ~~spectrophotometrically~~- spectrophotometrically at a wavelength of 590 nm.

1.6 Histological Evaluation of Liver Tissues

Haematoxylin and Eosin staining technique was used for the histological preparation of the liver tissues. After harvesting the tissues, the tissue sections were hydrated in descending grades of alcohol from 100%, 95%, 90% and finally 70% [13]. Each of the steps lasted for three (3) minutes and the tissues were washed in a running tap water and stained afterward with haematoxylin for twenty five (25) minutes, washed with water and then differentiated in acid alcohol. The tissues were then counter stained with eosin and blued in Scott water. Afterward, the tissues were hydrated in ascending grades of alcohol and cleared in xylene for three (3) changes in five (5) minutes each. The tissues were then mounted with cover slips using a mounting media and viewed under a light microscope and the photomicrographs taken at a magnification of x 250.

1.4 STATISTICAL ANALYSIS

Data obtained from the study were expressed as mean $\pm$ SEM. Statistical analysis was carried out using version 20 of SPSS with the aid of one way analysis of variance (ANOVA) and Tukey's post-hoc test. Values with ($P < 0.05$) were considered significant

2.0 RESULT

2.1 EFFECT OF ORAL ADMINISTRATION OF THEOPHYLLINE AND GLIBENCLAMIDE ON SERUM ALKALINE PHOSPHATASE (ALP) LEVEL ~~IN ALLOXAN INDUCED MALE DIABETIC WISTAR RATS.~~

In Fig 1 below the serum level of ALP was not significant in the theophylline (5 mg/kg and 10 mg/kg) treated groups; ($119.75 \pm 3.29 \mu\text{L}$ and $115.75 \pm 2.61 \mu\text{L}$) respectively, when compared to the diabetic control group. However, serum ALP was significantly higher in the theophylline treated groups compared to glibenclamide treated group. There was also a

significant decrease in the Glibenclamide treated group; $75.60 \pm 1.79 \mu/L$ when compared to the DC ($P < 0.05$)

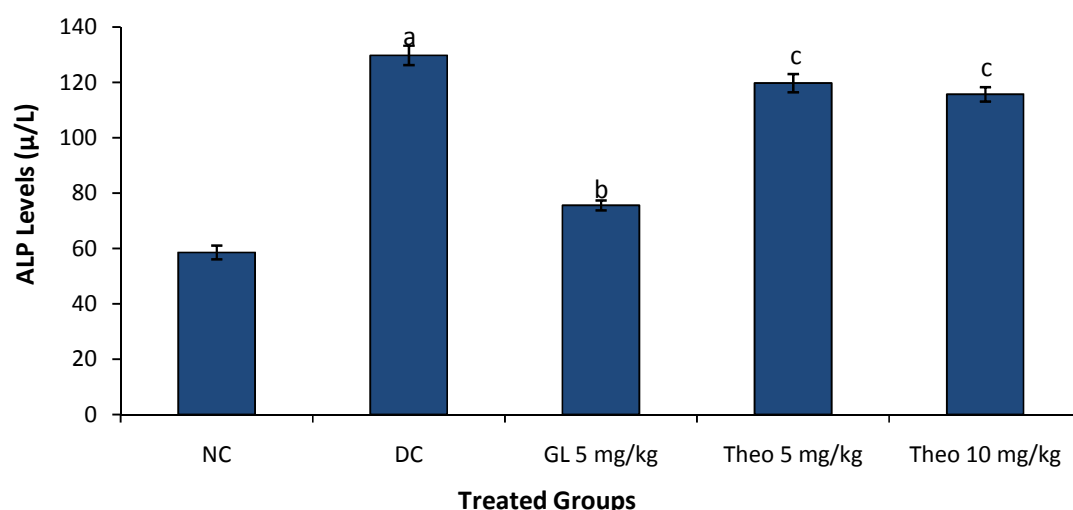


Figure 1: showing Serum ALP level following treatments with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant ($P < 0.05$) compared to normal control (NC), Superscripts a, b and c= statistically significant ($P < 0.05$) compared to groups 1, 2 and 3 respectively

2.2 EFFECT OF 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, although serum ALT was significantly higher in the theophylline treated groups when compared to the glibenclamide treated group. The level of ALT was also significantly lower ($P < 0.05$) in the Glibenclamide treated group; $39.80 \pm 2.06 \mu/L$ when compared to the DC.

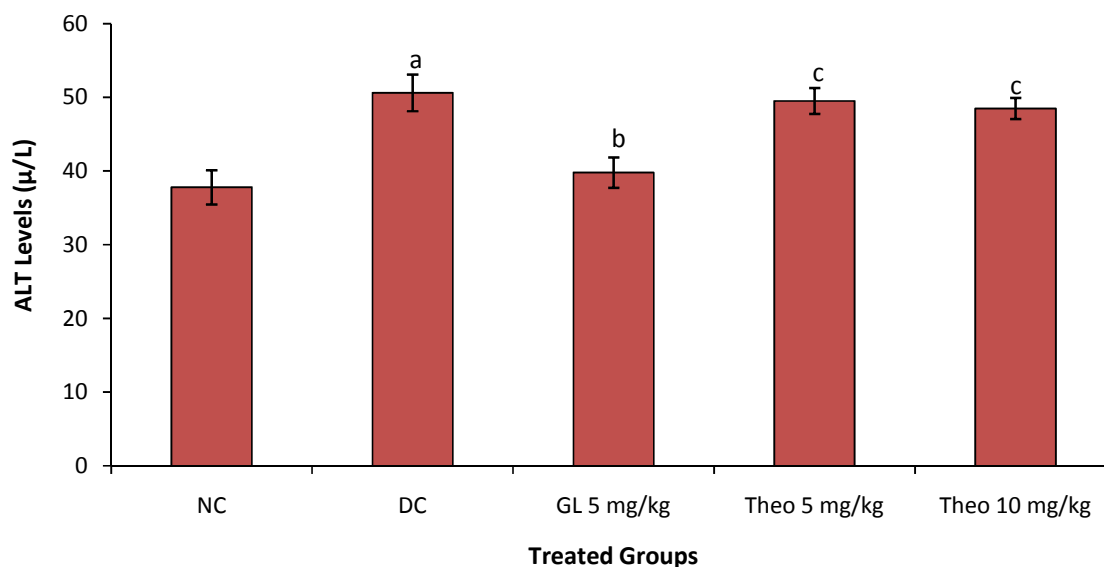


Figure 2: showing Serum level of Alanine amino transferase following treatments with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant ($P < .05$) compared to normal control (NC), Superscripts a, b and c= statistically significant ($P < 0.05$) compared to groups 1, 2 and 3 respectively

2.3 EFFECT OF 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 non-significantly lower 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 ($P < 0.05$).

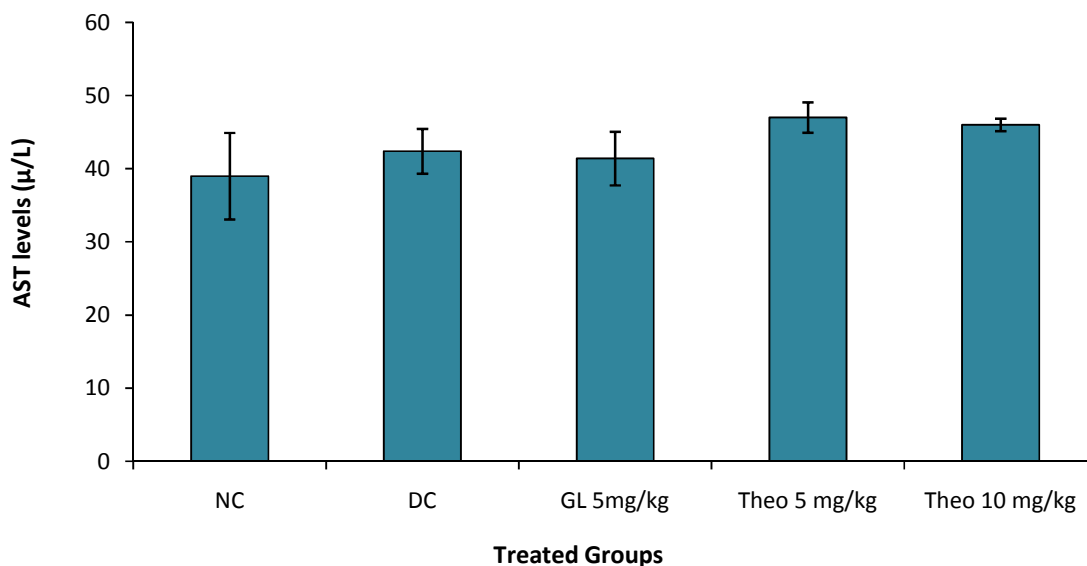


Figure 3: [showing Serum level of Alanine](#) amino transferase following treatment with glibenclamide and theophylline. NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide.

2.4 EFFECT OF 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 a mild decrease in fatty infiltration.

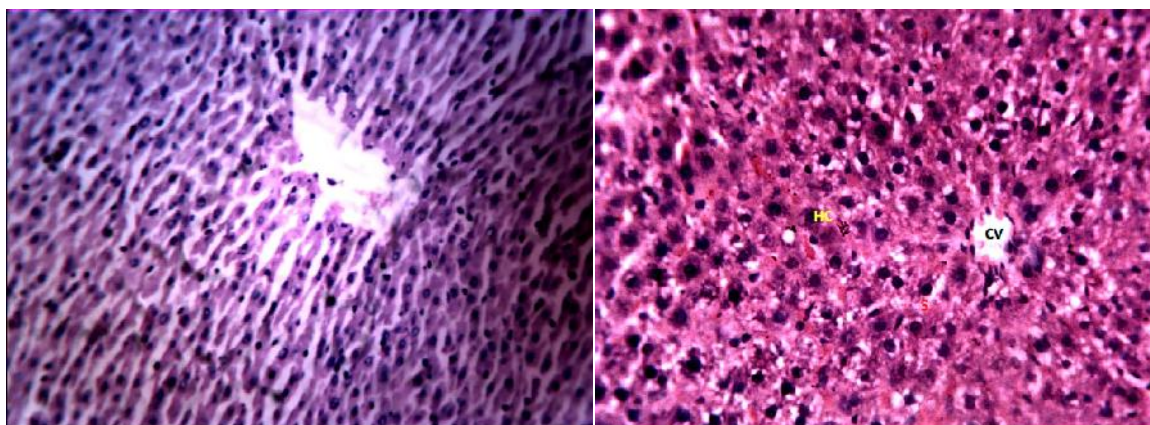


PLATE I: Diabetic control

Plate II: Glibenclamide (5 mg/kg)

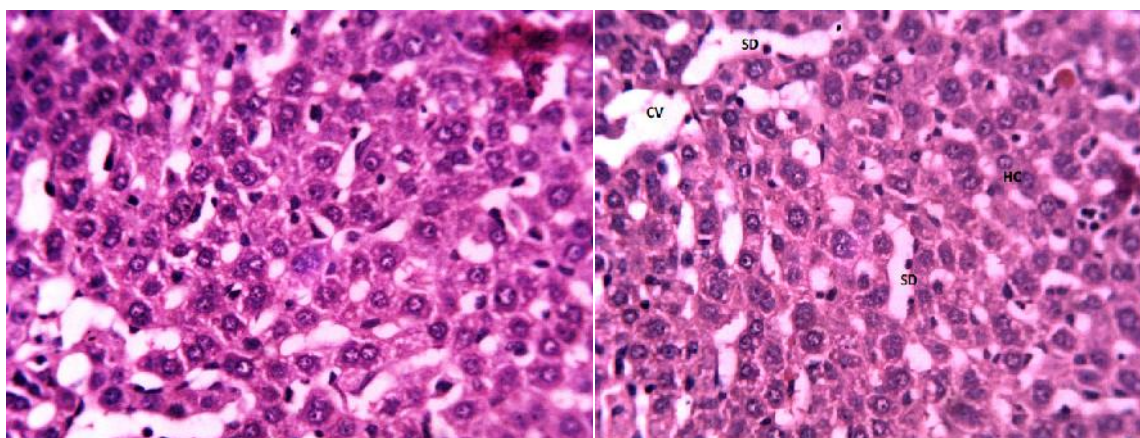


Plate III: Theophylline (5 mg/kg)

Pate IV: Theophylline (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. Increase in the liver enzymes reveals impaired liver function [14]. The decrease in the liver enzymes in theophylline treated groups could have been because of theophylline's coffee content which has been reported to decrease and elevated levels of liver enzymes [15]. Decaffeinated coffee intake lowers liver enzymes levels promoting liver health. The results of glibenclamide on serum liver enzymes from this study indicate hepatoprotective potential which is in concert to the result of the liver histomorphology. Histopathological evaluation of the liver tissues showed mild fatty changes in centrolobular portion of the liver in the diabetic rats. The Fatty infiltrations were reduced in the glibenclamide and 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 reduced desquamation of the hepatocytes along side restoration of a normal histoarchitecture relative to the diabetic control.

3.1 CONCLUSION

Glibenclamide treatment caused a reduced hepatotoxic activity by significantly lowering serum liver enzymes; ALP and ALT in contrast to theophylline which showed no significant differences in levels of serum enzymes in alloxan induced hyperglycaemic rats

3.2 Disclosure of conflict of Interest

The authors declare that there is no conflict of interest

3.3 Acknowledgments

The authors are grateful to the Faculty of medical sciences, Ahmadu Bello University for their support during this research.

3.2 REFERENCES

1. World Health Organization Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation, 2015; 32-42
2. Patel V, Sharma V. The Role of Natural Antioxidants in Oxidative Stress Induced Diabetes Mellitus. *Research Journal of Pharmaceutical Science*, 2014; 3(4): 1-6.
3. Henderson-Smart D, De Paoli A. Methylxanthine treatment for apnoea in preterm infants. *Cochrane Database of Systemic Reviews*, 2010; (3): 140-142
4. Seneff M, Scott J, Friedman B, Smith M. Acute theophylline toxicity and the use of esmolol to reverse cardiovascular instability". *Annals of Emergency Medicine*, 1990; 19(6): 671–3.
5. Szkudelski T. The mechanism of alloxan and streptozotocin action on β cells of the rat pancreas. *Physiological Research*, 2001; 50(6): 537-546
6. Weets I, DeLeeuw I, DuCaju M, Rooman R, Keymeulen B, Mathieu C, Rottiers R, Daubresse J, Rocour-Brumioul D, Pipeleers D. The incidence of type 1 diabetes in the age group 0-39 years has not increased in Antwerp (Belgium) between 1989 and 2000: evidence for earlier disease manifestation. *Diabetes Care*; 2002; 25:840–846
7. Marthins MI. *Zaria and its regions*. Department of Geography Ahmadu Bello University, Zaria, pp-41. 2006
8. Katsumata KY, Katsumata TO, Katsumata K. Potentiating effect of combined usage of three sulfonylurea drugs on the occurrence of Alloxan –induced diabetic rats. *Hormone Metabolism Research*; 1999; 25:125-12
9. Dhandapani S, Ramasamy SV, Rajagopal S, Namasivayam N. Hypolipidemic effect of *Cuminumcyminum* L. on alloxan-induced diabetic rats. *Pharmacological Research Journal*, 2002; 46(3): 251-255

- 231 10. Milani E, Shekouefeh N, Reza K, Mohammed J, Mohammed A. Reduction of diabetes-
232 induced oxidative stress by phosphodiesterase inhibitors in rats. *Comparative Biochemistry*
233 *and physiology Part C: Toxicology & pharmacology*; 2002; 140(2):251-255
- 234 11. Bowers GNJ, Mc-Comb RB. A continuous spectrophotometric method for measurement
235 the activity of serum alkaline phosphatase. *Clinical Chemistry*, 1966; 12:73- 78.
- 236 12. Bergmeyer H, Walefeld M. Méthode cinétique pour la détermination du TGO et TGP
237 sans phosphate de pyridoxal. *Clinica Chimica Acta*, 1978; 24-58
- 238 13. Laporta J, Peters TL, Merriman KE, Vezina CN Hernandez LL. Serotonin (5-HT) Affects
239 Expression of Liver Metabolic Enzymes and Mammary Gland Glucose Transporters during
240 the Transition from Pregnancy to Lactation. *PLoS ONE*, 2013; 8(2): 5784
- 241 14. Nikfar S, Khorasani R, Zamani J, Abdollahi M. Reduction of diabetes-induced oxidative
242 stress by phosphodiesterase inhibitors in rats. *Comp Biochem Physiol C Toxicol Pharmacol*,
243 2005;140(2): 251-5
- 244 15. Greer F, Hudson R, Ross R, Graham T. Caffeine ingestion decreases glucose disposal
245 during a hyperinsulinemic-euglycemic clamp in sedentary humans. *Diabetes*, 2001; 50:
246 2349–2354.