Original Research Article

ANTIOXIDANTS AND HAEMATOLOGICAL EFFECTS OF CRUDE EXTRACT OF STAR FRIUT (*Averrhoa carambola*) LEAVE IN ALLOXAN-INDUCED FEMALE DIABETIC ALBINO RATS

ABSTRACT

Various haematological and antioxidant parameters have aberrant values as diabetic problems worsen, and the toxic side effects of some medications used to treat diabetes have also contributed to the abnormal values of these parameters. Natural remedies derived from plants are frequently praised for being accessible, inexpensive, and safe compared to allopathic medications. This study evaluated the efficacy of 80% of methanol leaf extract of *Averrhoa carambola* (MEAC) in alloxan diabetic rats. Thirty female albino rats were allotted into six groups. Group 1 (Normal control), group 2 (Negative control), group 3 (Positive/Glibenclamide), group 4 (animals treated with 100mg/kg of MEAC extract), group 5 (animals treated with 200mg/kg of MEAC extract), group 6 (animals treated with 400mg/kg of MEAC extract) respectively for 28 days. After treatments, antioxidant and haematological parameters were determined. This study showed no significant difference in the activity of Glutathione (GSH) between the diabetic and normal control animals. Treatment of the animals with MEAC also did not affect their GSH enzymes. The diabetic control animals exhibited a significantly ($p < 0.05$) reduced catalase (CAT) enzyme activity compared to the normal control animals. Treatment of the diabetic control animals with MEAC at all doses increased catalase activity to levels significantly higher than the normal control animals and the diabetic animals. Malondialdehyde (MDA) was significantly ($p < 0.05$) elevated in the diabetic control animals compared to the normal rats. Treatment with MEAC exhibited a significant dose-dependent decrease in MDA level compared to the diabetic control animals. Total White Blood Cells (TWBC), Hemoglobin (Hb), Red Blood Cells (RBC), and Packed Cell Volume (PCV) showed a significant ($P< 0.05$) reduction in the diabetic animals. Treatment with MEAC extracts significantly ($P< 0.05$) improves the levels of these indices in diabetic animals. The findings imply that *Averrhoa carambola* leaf extracts are risk-free, effective at treating some biochemical and haematological abnormalities linked to diabetes mellitus and hence might be suggested as a supplement to dietary therapy.

KEYWORDS: ***Alloxan monohydrate; Averrhoa carambola; Diabetes; Glibenclamide; Hematology; oxidative stress.***

1. INTRODUCTION

Diabetes Mellitus (DM) has been classified as a metabolic disorder characterized by an elevated blood glucose level (hyperglycemia), which may be due to a lack of insulin or a drastic decrease in insulin effectiveness. Among the symptoms are hyperglycemia-related fatigue, weight gain, polyphagia, and polyuria [1]. According to the World Health Organization, there will be 150% more individuals with diabetes in the next 25 years, and a greater percentage of those affected will be children and young adults [2]. The chance of acquiring both short-term and long-term problems from diabetes is increased by hyperglycemia. Cardiovascular disease (CVD), nephropathy, retinopathy, and neuropathy are the most prevalent long-term consequences of diabetes [3,4]. Adult blindness, non-traumatic lower limb amputation, and end-stage renal failure are all most commonly caused by diabetes. [5]. Cells in healthy people use oxygen to make energy, but the process also creates free radicals, which are harmful byproducts that harm DNA and proteins. The body naturally creates antioxidants to scavenge those free radicals and lessen oxidative tissue damage. A system of defenses, including antioxidant enzymes (SOD, catalase, glutathione peroxidase), small molecules with scavenging capabilities, such as antioxidant vitamins (A, C, and E), reduces or prevents oxidative damage [6].

Oxidative stress is a pathological situation that results from an imbalance between the elimination and production of free radicals. It is noteworthy that oxidative damage, which builds up throughout a person's life and has been linked to ageing and age-related diseases like cancer,

heart disease, and neurodegenerative disorders, among others, can be caused if the production of free radicals exceeds the antioxidants' protective effects [6]. It has been shown that long-term diabetes alters the equilibrium between the generation of reactive oxygen species and the overall antioxidant state [7]. However, additional work has to be done on the status of each antioxidant enzyme in diabetes, particularly in the early stages of the disease.

Averrhoa carambola, also known as Star fruit in Asia, or kpakpando mkpuru in Igbo, is a member of the Oxalidaceae family. It is an old plant with many uses and contains secondary metabolites with various biological activities [8]. *A. carambola* has been identified to have parts that can be used medicinally. It has been suggested that a carambola leaf decoction can be used to treat diabetes [9]. *A. carambola's* stems have been demonstrated to have selective action against brain tumour cells, but the leaves have been proven to have activity against liver cancer cells [10]. The guinea pig's atrial inotropic is suppressed by the aqueous leaf extract of *A. carambola* [11]. The anti-inflammatory [12], analgesic [13], hypoglycemic [14], anthelmintic [15], anti-ulcer [16], hypotensive [17], antioxidant [18], hypercholesterolaemia and hypolipidemic, [15], antimicrobial [12], and antitumor [10] properties of *A. carambola* plant parts have also been highlighted in a number of studies. Significant phytochemicals include tannins, saponins, alkaloids, and flavonoids have also been reported in *A. carambola* plant parts [19].

This study was set up to evaluate the potency of the leaf extract on induced diabetic rats. Specifically, the study sort: to determine the effect of the leaf extract on the glucose level of the diabetic rats, determine the changes in haematological parameters (PVC, RBC, and WBC) in induced diabetic rats, and investigate the effect of the extract on the antioxidant parameters (CAT, GSH, MDA) in the experimental rats. This work is important because biochemical and haematological changes are major observable clinical and pathological features common with diabetes.

2. MATERIALS AND METHODS

Chemical and Reagents

Alloxan monohydrate (Sigma Aldrich Chemicals, USA) induced diabetes. All other chemicals and reagents used were of analytical grades and products

Plant Materials

Star fruit (*Averrhoa carambola*) fresh leaves were plucked from a healthy tree at Lodu Ndume Ahiaeke in Umuahia North Local Government of Abia State. The plant was authenticated by a Taxonomist (Dr Ibe K. Ndukwe) from the forestry department, College of Natural Resources and Environment Management (CNREM), Michael Okpara University of Agriculture Umudike (Specimen voucher number = IHF 25162). The leaves were washed and dried at room temperature; it was weighed and milled into powdered form 250g. The powdered leaves were soaked in methanol and distilled water in the ratio of 80:20 respectively and left to stand for 3 days with occasional shaking. This was filtered using Whatman No.1 filter paper, and the filtrate was subsequently evaporated to obtain the dry matter; a rotary evaporator was used under reduced pressure at 40^{o}C.

Experimental Animals

Thirty (30) healthy female albino rats weighing 95-125g procured from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, were used for the study. The animals, on arrival, were weighed to obtain initial weight and were acclimatized for 14 days in the animal house of the Biochemistry Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike; The animals were allowed access to standard food and water till the end of the research which lasted for 28 days.

Ethical consideration

Throughout the experiment, all the rats were housed at 25^{o}C in clean metal cages under normal daylight humid conditions. The rats were freely fed pellets, given tap water, and made available throughout the experiment as approved by the departmental committee on animal use guidelines, Michael Okpara University of Agriculture, Umudike on handling experimental animals.

Experimental Design and Animal Grouping

Rats were divided into six groups of six rats each: Group 1 (Normal control), group 2 (Negative control), group 3 (Positive/Glibenclamide), group 4 (animals treated with 100mg/kg of MEAC

extract), group 5 (animals treated with 200mg/kg of MEAC extract), group 6 (animals treated with 400mg/kg of MEAC extract) respectively.

Table 1: Treatment Details

Group		Treatment
1	Normal Control	Feed + H_2O ad libitum
2	Negative (Untreated) control	Alloxan + feed+ H_2O ad libitum
3	Positive control (2mg/kg bw)	Alloxan + standard drug (Glibenclamide) +feed+ H_2O
4	Diabetic-treated rats (100mg/kg of MEAC extract)	Alloxan + 100ml/kg extract of MEAC + feed+ H_2O ad libitum
5	Diabetic-treated rats (200mg/kg of MEAC extract)	Alloxan + 200ml/kg extract of MEAC + feed+ H_2O ad libitum
6	Diabetic-treated rats (400mg/kg of MEAC extract)	Alloxan + 400ml/kg extract of MEAC + feed+ H_2O ad libitum

Sacrifice and Sample Collection
After the experiment, Blood samples were collected through cardiac puncture under anaesthesia into an EDTA bottle. Pooled blood sample (1 ml per rat, 9 ml per treatment) was used for biochemical analysis.

Determination of Biochemical Parameters
The plasma samples were used for the determination of Malondialdehyde (MDA) [20] as the lipid peroxidation indicator, Catalase (CAT) [21], and Glutathione (GSH) as the antioxidant enzymes.

Determination of Haematological Parameters
Haematological parameters were analyzed using a haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA.) following the methods of Chhabra [22]. The parameters assayed were as follows: white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), and packed cell volume (PCV)

Statistical Analysis
Statistical data analysis was carried out with SPSS version 22.0 using One Way Analysis of variance (ANOVA). The statistical analysis data were reported as Mean ± standard deviation (SD). Significant difference using Tukey's Post Hoc test was accepted at 95% confidence level of probability, i.e., if $p<0.05$

3. RESULTS

Table 2: Comparison of Antioxidant levels treated with Star fruit (*Averrhoa carambola)* at different doses

Group	Treatment	GSH (µ/L)	MDA (mmol/l)	CAT (µ/L)
1	Normal Control	45.04 ± 0.20	0.44 ± 0.31	10.45 ± 1.05
2	Negative (Untreated) control	40.08 ± 0.21	12.66 ± 4.03	8.25 ± 0.03
3	Positive control (2mg/kg bw)	43.04± 0.11	0.83± 0.45*	10.62 ± 0.15
4	Diabetic treated rats (100mg/kg of MEAC extract)	45.07 ± 0.02	0.70± 0.13*	11.22± 0.52*
5	Diabetic treated rats (200mg/kg of MEAC extract)	44.04± 0.21	0.73± 0.12*	11.08± 0.04*
6	Diabetic treated rats (400mg/kg of MEAC extract)	44.06± 0.01	0.71± 0.74*	11.01± 0.21*

Values are expressed as mean ± SD (n = 6). *$p<0.05$ when compared with the negative control
Abbreviation: GSH: Glutathione; MDA**:** Malondialdehyde; CAT: Catalase; MEAC: Methanol Extract of *Averrhoa carambola*

The result of antioxidant markers in healthy and alloxan-induced diabetic animals is presented in Table 2. There was no significant difference in the activity of GSH between the diabetic animals and normal control animals. Treatment of the animals with MEAC also did not affect their GSH enzymes. The diabetic control animals exhibited a significantly ($p <0.05$) reduced catalase

enzyme activity compared to the normal control animals. Treatment of the diabetic control animals with MEAC at all doses increased catalase activity to levels significantly higher than the normal control animals and the diabetic animals. The oxidative stress marker, MDA, was significantly ($p < 0.05$) elevated in the diabetic control animals compared to the normal rats. The diabetic animals treated with MEAC exhibited a significant dose-dependent decrease in MDA level compared to the diabetic control animals.

Table 3: Effect of Haematological indices of alloxan-induced diabetic albino rats treated with methanol extract of Star-fruit (*Averrhoa Carambola*) leaves

Groups	Treatment	RBC (g/dl)	TWBC (g/dl)	Hb (g/dl)	PCV (g/dl)
1	Normal Control	154.15 ± 0.10	70.15 ± 2.10	11.54 ± 3.05	50.15 ± 1.05
2	Negative (Untreated) control	126.12 ± 0.40	33.60 ± 3.30	7.73 ± 0.22	30.18 ± 2.24
3	Positive control (2mg/kg bw)	157.10 ± 0.14*	65.05 ± 4.03*	11.64 ± 1.42*	54.16 ± 4.31*
4	Diabetic treated rats (100mg/kg of MEAC extract)	160.16 ± 1.14*	70.64 ± 1.40*	12.15 ± 1.43*	55.26 ± 1.43*
5	Diabetic treated rats (200mg/kg of MEAC extract)	169.20 ± 5.06*	79.18 ± 1.05*	13.41 ± 2.31*	56.10 ± 2.19*
6	Diabetic treated rats (400mg/kg of MEAC extract)	174.23±3.11*	83.05±0.14*	14.52±0,25*	57.31±0.26*

Values are expressed as mean ± SD (n = 6). *p<0.05 when compared with the negative control
Abbreviation: TWBC: Total White Blood Cells; Hb: Hemoglobin; RBC: Red Blood Cells; PCV: Packed Cell Volume; MEAC: Methanol Extract of *Averrhoa carambola*

The effect of MEAC extract on haematological indices of healthy and diabetic animals is presented in Table 3. Our findings showed a significant ($P< 0.05$) reduction in TWBC, HB, RBC and PCV in the diabetic animals. Treatment with MEAC extracts significantly ($P< 0.05$) improves the levels of these indices in diabetic animals.

4. DISCUSSION

In the pathophysiology of diabetes mellitus, hyperglycemia produces reactive oxidants through several mechanisms, including the polyol pathway and the auto-oxidation of glucose. This impairs insulin secretion and glucose uptake [23]. In diabetes situations, antioxidant indicators are also decreased, which increases oxidative stress [24]. In this study, an increase in the production of reactive oxygen species is responsible for the elevated levels of MDA, a secondary marker of lipid peroxidation, in diabetic rats (ROS). Lipid breakdown and the harmful clinical symptoms connected to diabetes mellitus can result from excessive lipid peroxidation [25]. The ability of the formulation to suppress lipid peroxidation is suggested by the fact that treating diabetic rats with MEAC extracts corrected the MDA levels. The body's fight against ROS depends heavily on antioxidant enzymes. Glutathione (GSH) catalyzes the reduction of H_2O_2 by oxidizing to GSSG. Superoxide dismutase (SOD) is involved in scavenging superoxide anions and converting them to less reactive molecules. After that, a catalase enzyme detoxifies H_2O_2, turning it into water and oxygen, and a reductase enzyme regenerates GSH. When given to diabetic animals, alloxan decreased the activity of several enzymes. The fact that GSH reduces the oxidative stress associated with diabetes may account for the drop in GSH levels in diabetic rats. MEAC extracts reversed the changes in GSH and CAT by raising their concentrations in the diabetic rats. This suggests that MEAC extracts can scavenge and can inhibit oxidative stress.

Erythrocytes have been shown to significantly deliver oxygen to body tissues throughout systemic circulation [26]. Hyperglycemia in diabetes mellitus has been demonstrated to impair

the deformability of red blood cells, which are otherwise deformed without rupturing as they withstand continuous flow conditions through narrow capillaries. This impairment results from the generation of reactive oxygen species observed in diabetes mellitus. This follows the finding of this study that showed a significant reduction in TWBC, HB, RBC, and PCV in the diabetic animals. The decrease in WBC, Hb, RBC and PCV values observed after administration of alloxan may be due to abnormal haemoglobin synthesis, failure in blood osmoregulation and plasma osmolarity [27]. The level of RBCs and their related indices were appreciably improved as the extract was given. This gives credence to the ability of the leaf extract to stimulate the formation/secretion of erythropoietin, which triggers stem cells in the bone marrow to produce red blood cells [28].

In the same way, WBC, Hb, PCV, and RBC significantly increased ($p<0.05$) in all MEAC extracts treated groups compared with the untreated diabetic and the positive control rats. Various haematological indices and the immune system have been altered during diabetes [29]. Ajagbonna et al. [30] reported the alteration of normal haematological and biochemical values of rats treated with extract of *Calotropis procera* W. T. Aiton (Gentianales: Apocynaceae).

5. CONCLUSION AND RECOMMENDATION

The administration of MEAC extracts has been found to have antioxidant activities at 100, 200 and 400 mg/Kg-1 body weight doses. In this study, the reversal of the impact of diabetes on the haematological and several biochemical parameters was linked to the antioxidant capabilities that might be related to the high density of phytonutrients. Therefore, it can be concluded that MEAC extracts are safe and effective at normalizing the haematological abnormalities linked to diabetes mellitus. As a result, they may be suggested as a supplement to dietary therapy for diabetes. It is highly advised to characterize the solvent fraction to identify the bioactive chemical or component responsible for this activity.

ETHICAL APPROVAL

All authors with this declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the College of Natural Sciences, Michael Okpara University of Agriculture (MOUAU) Research and Ethics Committee.

CONFLICT OF INTEREST

I declare that the authors had no conflict of interest. All figures presented in this manuscript are original and have not been used or presented elsewhere

REFERENCES

1. Prince OA, Chinwe EO, Chiemeziem AO, Chimaraoke O, Peter OE, George C.N. Hypoglycemic effect of methanol extract of pawpaw (*Carica papaya*) leaves in alloxan-induced diabetic rats. *Intl J Innov Sci Res Tech*. 2022; 7(1); 627-631. https://doi.org/10.5281/zenodo.5995940
2. King HR, Aubert E, Herman WH. Global Burden of diabetics (1995-2005) Prevalence, Numerical Estimation and Projection. *Diabetics care;* 1998; Vol. 21: 1411- 11431.
3. Prince OA, Chimaraoke O, Chinwe EO, George CN, David UI, Peter OE. Hepato-Protective Effect and Lipid Profile of Honey on Alloxan-Induced Diabetic Rats. *Asian J Res Biochem.* 2022; 10(1): 16-24. https://doi.org/10.9734/ajrb/2022/v10i130212
4. Ogbonna HN, Nwankpa UD, Aloh GS, Ibeh RC. Effect of Methanol Extract of Unripe *Carica papaya* Pulp on Lipid Profile and Liver Function of Alloxan-Induced Diabetic Rats. *Intl J Biochem Res & Rev.* 2020; 29(4), 1-11. https://doi.org/10.9734/ijbcrr/2020/v29i430180
5. Bredleharm HW. Effect of an Anti-diabetic extract of catharanthos on enzyme activities in Alloxan-induced diabetic rats. *Journal of Ethno pharmacological Center for Diabetes Control and Prevention* 4th Ed. 2010; 45-57
6. Alaebo PO, Umeh VA, Njoku GC, Ezennaya IE, Anyadike NN, James UA, Ezeh CJ, Paschal U. Antioxidant and Nephroprotective Effects of Honey in Alloxan-Induced Diabetic Rats. *J Appl Life Sci Intl.* 2022; 25(2), 26-32. https://doi.org/10.9734/jalsi/2022/v25i230285

7. Ibeh RC, Ogbonna HN, Aloh GS, Nwuke CP, Ikechukwu GC, Eziefuna OE, Dialah DO, Usuka IC, Nkemjika KP, Ezerioha CC. Hepatoprotective, Antioxidant and Hypolipidemic Potentials of *Mucuna pruriens* in a Diabetic Experimental Animal Model. *Intl Res J Gastroente Hepatol*, 2020; 3(3), 20-29. Retrieved from https://journalirjgh.com/index.php/IRJGH/article/view/30122
8. Muthu N, Lee SY, Phua KK, Bhore SJ. Nutritional, medicinal and toxicological attributes of star-fruits (*Averrhoa Carambola L.):* a review. *Bioinformation*, 2016; 12(12), 420.
9. Provasi M, Oliveira CE, Martinho MC, Pessini LG, Bazotte RB, Cortez DAG. Avaliação da toxicidade e do potencial anti-hiperglicemiante da *Averrhoa carambola L.* (Oxalidaceae). *Acta Scientiarum;* 2001; 23, 665-669.
10. Tadros SH, Sleem AA. Pharmacognostical and biological study of the stem and leaf of *Averrhoa carambola* L. Bull Faculty of Pharmacology, 2004; 42, 225-246.
11. Vasconcelos CM, Gondim AN, Cruz JS, Mafra RA, Silva BA, Conde-Garcia EA. Aqueous leaf extract of *Averrhoa carambola L.* (Oxalidaceae) reduces the inotropic effect of BAY K 8644 on the guinea pig atrium and the calcium current on GH3cells. *Revista Brasileira de Farmacognosia,* 2008; 18(4), 539-543.
12. Sripanidkulchai B, Tattawasart U, Laupattarakasem P, Wongpanich V. Anti-inflammatory and Bactericidal Properties of elected Indigenous Medicinal Plants Used for Dysuria. *Thail J Pharm Sci*. 2002; 26(12), 33-38.
13. Das BN, Ahmed M. Analgesic activity of the fruit extract of *Averrhoa carambola*. *Int J Life Sci Biotech Pharm Res*. 2012; 1(3), 34-37
14. Gunasekara LCA, Fernando PHP, Sivakanesan R. A preliminary study on the hypoglycaemic effect of *Averrhoa carambola* (Star fruit) in rats. Proceedings of the Peradeniya University Research Sessions, Sri-Lanka, 2011; 83, 2011.
15. Chau CF, Haung YL, Lee MH. Insoluble fibre-rich fractions derived from Averrhoa carambola: Hypoglycaemic effects determined by in vitro methods. Lebensm-Wiss Technology; 2004; 37: 331-335.
16. Shah NA, Raut BA, Baheti A, Kuchekar BS. In-vitro Anthelmintic activity of leaf extract of *Averrhoa carambola* against Pheretima Posthuma. Pharmacogyonline; 2011; 1, 524-527.
17. Goncalves ST, Baroni S, Bersani-Amado FA, Melo GA, Cortez DA, Bersani-Amado CA, Cuman RK. Preliminary studies on gastric anti-ulcerogenic effects of *Averrhoa carambola* in Rats. *Acta Farmaceutica Bonaerense*, 2006; 25(2), 245-7
18. Soncini R, Santiago MB, Orlandi L, Moraes GO, Peloso ALM, dos Santos MH, Giusti-Paiva A. Hypotensive effect of aqueous extract of *Averrhoa carambola L.* (Oxalidaceae) in rats: An in vivo and in vitro approach. *J ethnopharma*, 2011; 133(2), 353-357.
19. Nettem S, Eswaraiah MC, Dipankar B. Evaluation of in-vitro antioxidant activity of *Averrhoa carambola* stem ethanolic extract. *Intl J PharmTech Res*, 2013; 5(4), 1611-1618.
20. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colourimetric method. ClinicaChimica Acta. 1978;90(1):37-43. DOI:10.1016/0009-8981(78)90081-5]
21. Aebi H. Catalase In vitro. *Methods enzymol.* 1984; 105:121-6. DOI:10.1016/S0076-6879(84)05016-3
22. Chhabra G. Automated haematology analyzers: recent trends and applications. *J Lab Physi*, 2018; 10(01): 015 – 016.
23. Ighodaro OM. Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomed. Pharmacother*. 2018; 108, 656–662
24. Burgos-Morón E, Abad-Jiménez Z, Marañón AM, Iannantuoni F, Escribano-López I, López- Domènech S, Salom C, Jover A, Mora V, Roldan I. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. *J. Clin. Med*. 2019; 8, 1385
25. Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*. 2019; 8-23
26. Waggiallah H, Alzohairy M. "The effect of oxidative stress on human red cells glutathione peroxidase, glutathione reductase level, and prevalence of anaemia among diabetics," *North Amer J Med Sci*, 2011; vol. 3, no. 7, pp. 344– 347.
27. Stookey JD, Burg M, Sellmeyer DE, Greenleaf JE, Arieff A, Van Hove L, Gardner C, King JC. A proposed method for assessing plasma hypertonicity in vivo. *Eur J Clin Nutr*. 2007; 61(1): 143 – 146.

28. Lodish H, Flygare J, Chou S. From stem cell to erythroblast: regulating red cell production at multiple levels by multiple hormones. *IUBMB Life*, 2010; 62(7): 492 – 496
29. Mansi K, Lahham J. Effects of *Artemisia sieberi Besser* (A. herba-alba) on heart rate and haematological values in normal and alloxan-induced diabetic rats. *Austr J Basic and Appl Sci*. 2008; 4(2): 57 – 62
30. Ajagbonna OP, Onifade KI, Suleiman U. Haematological and biochemical changes in rats given extract of *Calotropis procera*. *Sokoto J Vet Sci.* 1999; 1(1): 36 – 42.