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Effect of Aqueous Leaf Extract of *Desmodium salicifolium* on Renal and Haematological Indices of Diabetic Wistar Rats

Momoh T. B[1], Abaniwo R. M[2], Hassan A. O[3], Ojade F. E[4], Shaibu V. N[4]

[1]Department of Plant Science and Biotechnology, Faculty of Natural Sciences, Kogi State University, Anyigba, Kogi State, Nigeria

[2]Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Kogi State, Nigeria

[3]Department of Space Regulations and Special Duties, National Space Research and Development Agency, Nigeria

[4]Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic, Lokoja, Kogi State, Nigeria

Corresponding author: MOMOH Theophilus Boniface

E-mail: *theophilusmomoh@rocketmail.com*

Abstract

This study investigated the effect of the aqueous leaf extract of *Desmodium salicifolium* on hematological and renal function parameters of diabetic rats. The study was carried out using alloxan model of diabetes. All treatments were carried out orally for 28 days and at the end of the treatment, rats were sacrificed and blood samples collected for the estimation of haematological and renal function parameters. Results showed that white blood cell (WBC) count was significantly ($p< 0.05$) increased while red blood cell (RBC) and packed cell volume (PCV) were significantly ($p< 0.05$) decreased in diabetic rats. Treatment with the aqueous- leaf extract of *Desmodium salicifolium* ameliorated the imbalances in hematological parameters caused by alloxan. Treatment with the extract had no significant ($p> 0.05$) effect on the serum electrolytes. However, serum level of urea was significantly increased following treatment with extract. It was concluded that the extract could play a key role in the management haematological abnormalities associated with diabetes but its effect on renal function needs further investigation.

Keywords: *Desmodium salicifolium*, Renal, Haematological, Diabetic, Wistar Rats

1.0. Introduction

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (Ugochukwu *et al.*, 2003), lipoprotein abnormalities (Scoppla *et al*., 2001), raised basal metabolic rate (Nawata *et al*., 2004; Okwu *et al.*, 2006), defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (Unwin *et al.*, 2001; Idakwoji and Uzuazokaro, 2018). The hyperglycaemia in diabetes mellitus can result from an absolute deficiency in insulin secretion (type 1 DM), insulin action (type 2 DM) or both (Ahmed and Goldstein, 2006; Andrade-Cetto *et al*., 2007).

Haematological changes associated with diabetes consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazz *et al.*, 2004). This is presented by alteration in platelet count and activity, coagulopathy, fibronolytic aberration, haemorrhagic factors and changes in endothelial metabolism (McFalarnce, 1997). The underlying cause of the

changes in the hematology in diabetes mellitus is mainly due to oxidative damage (McFalarnce, 1997).
The elevation of renal function markers such as serum electrolytes, creatinine and urea levels observed in diabetes could be traceable to the thickening of glomerular and tubular basal membranes, with progressive mesangial expansion (diffuse or nodular) leading to progressive reduction of glomerular filtration surface (Zelmanovitz *et al.*, 2009). This is typical of diabetic nephropathy which is one of the major complications of diabetes mellitus. It is characterized by frequent microalbuminuria, elevated arterial blood pressure, a persistent decline in glomerular filtration rate and a high risk of cardiovascular morbidity and mortality (Raja and Shaker, 2012). The two main risk factors for diabetic nephropathy are hyperglycemia and arterial hypertension, but the genetic susceptibility in both type 1 and type 2 diabetes is of great importance (Zelmanovitz *et al.*, 2009).

A need exists to search for agents that tackle these pathophysiological aspects of diabetes. This study therefore sought to explore the potential beneficial effects of aqueous leaf- extract of *Desmodium salicifolium* on haematological and renal function parameters of diabetic wistar rats.

2.0. Materials and Methods

2.1 Materials

2.1.1 Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor. Chlorpropamide (250mg) was purchased from a local pharmacy shop.

2.1.2 Animals

Male adult Wistar rats of weighing 150–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

2.2 Methods

2.2.1 Plant Collection and Identification

The leaves of *Desmodium salicifolium* were collected from a natural habitat in Okpella area of Kogi State, Nigeria. The plants were identified at the herbarium unit of Biological Sciences Department, Federal University, Lokoja and voucher specimens were deposited for future references.

2.2.2 Preparation of Extracts

The leaves of *Desmodium salicifolium* were shade-dried for five (5) days and pulverized using an electric blender. One thousand (1000) gram of the pulverized leaves was soaked in distilled water for 72- hours. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract (henceforth reffered to as DSAE) was concentrated using a free- dryer.

2.2.3 Acute Toxicity Study

The oral median lethal dose (LD50) of the extract was determined in rats according to the method of Lorke (1983).

2.2.4 Experimental Design

2.2.4.1 Induction of diabetes

Diabetes was induced in adult male albino rats according to the method of Dunn and Mc Letchie (1943). The animals were fasted overnight and administered intraperitoneally 150 mg/ kg Alloxan monohydrate**.** After 72 h of administeration, rats having Fasting Blood Sugar (FBS) >200 mg/dl were considered hyperglycaemic and hence diabetic and used for the study.

2.2.4.2 Grouping of animals/ Treatment

Twenty-five (25) adult male albino rats were divided into 5 groups of 5 animals each and treated as follows:

Group 1: Diabetic control and received 1ml Normal saline
Group 2: Diabetic and received 250mg/ kg Chlorpropamide
Group 3: Diabetic and received 200 mg/ kg DSAE
Group 4: Diabetic and received 350 mg/ kg DSAE
Group 5: Diabetic and received 500 mg/ kg DSAE

All treatments were carried out orally for 28 days.

Fasting Blood Sugar (FBS) of the rats was monitored weekly during the period of treatment using Fine Test® glucometer and its corresponding strips. At the end of the 28- day treatment, the animals were anaesthetized under chloroform vapour and sacrificed. Blood

samples were obtained by cardiac puncture and poured into EDTA sample bottles. The samples were used for analysis within 12 h of collection.

2.2.4.3. Haematocrit determination

The packed cell volume (PCV) was estimated using the method of Alexander and Griffiths (1993). Haematocrit capillary tubes were filled by capillary action to mark with whole blood and bottom end of the tubes were sealed with plasticine. The tubes were centrifuged for 5 min using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along the haematocrit reader until the meniscus of the plasma intersects the 100% line.

2.2.4.4. Haemoglobin estimation

Cyamethaemoglobin (Drabkin) method (Alexander and Grifiths, 1993) of haemoglobin estimation was employed. Twenty microlitres of EDTA anticoagulated whole blood was added to 5 ml of Drabkin reagent mixed and incubated for 5 min at room temperature for the colour to develop. The absorbance was read against reagent blank at 540 nm using optima SP-300 Spectrophometer.

2.2.4.5. Total white blood cell count

The estimation of total white blood cells was done by visual method using New Improved Neubauer counting chamber. A 1 in 20 dilution of whole blood was made in Turk's fluid and the counting chamber with its cover glass already in position was filled with the diluted blood using a Pasteur pipette and ensuring that the chamber was filled in one action. The charged chamber was allowed to remain undisturbed for 2 min for the cells to settle. The cells were then counted microscopically using x40 objective lens. Four squares at the corners of the chamber were counted and the result was expressed in cells per litre of whole blood.

2.2.4.6. Red blood cell count

Red blood cells were counted by visual method using new improved Neubauer counting chamber. A 1 in 200 dilution of blood was made in formol citrate solution (Haymen's fluid) and the counting chamber with its cover glass in position was filled with the diluted blood using Pasteur pipette and ensuring that the chamber was filled in one action. The chamber was allowed to settle for 2 min for the cells to settle. Five squares, the four corners and the central squares were counted using x40 objective lens.

2.2.4.7 Determination of serum creatinine and urea level

Determination of serum urea level was conducted based on the 2, 3 diacetyl monoxime method using thiosemicarbazide and serum creatinine was determined by the Rehberry Method.

2.2.4.8 Determination of serum electrolytes

Serum sodium, potassium and phosphate were determined using the flame photometric method while serum chloride was determined using the mercuric nitrate titrimetric method.

2.2.5. Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Duncan test and difference between means at $P > 0.05$ were considered significant.

3.0. Results

The results of acute toxicity studies showed no mortality nor physical changes, changes in respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of aqueous extract of *Desmodium salicifolium.* The oral LD_{50} of the extract was then taken to be > 5000 mg/kg. The effect of the extract on the haematological parameters of diabetic Wistar rats is presented in Table 1. Administration of alloxan significantly ($P<0.05$) increased and decreased WBC and RBC count respectively. The results showed that the extract dose dependently and significantly ($P<0.05$) decreased the WBC count and increased the RBC count of rats. Treatment with the extract had no significant ($P<0.05$) effect on the hemoglobin level and platelet count of the rats. Treatment with the extract significantly ($P<0.05$) increased the urea level and significantly ($P<0.05$) decreased the serum creatinine level. The extract at all doses had no significant ($P<0.05$) effect on the serum electrolytes of the rats (Figures 1 and 2).

Table 1: Effects of Aqueous Leaf- Extract of *Desmodium salicifolium* on Serum Haematological Parameters of Diabetic Rats

Treatment	WBC ($x10^9$/L)	RBC ($x10^9$/L)	Hb (g/L)	PCV (%)	Plat ($x10^9$/L)
Group 1	17.3±2.44[b]	139.3±15.44[a]	09.8±0.44[a]	33.2±2.44[a]	906.4±52.16[a]
Group 2	14.4±1.33[ab]	140.5±18.21[a]	10.3±0.30[a]	42.3±2.67[b]	900.1±54.66[a]
Group 3	14.3±1.17[ab]	136.4±20.14[a]	10.1±0.26[a]	44.6±2.45[b]	903.4±43.81[a]
Group 4	12.2±2.12[ab]	178.2±17.12[b]	10.0±0.31[a]	45.6±3.19[b]	908.3±41.53[a]
Group 5	12.6±1.43[a]	185.4±14.21[b]	10.2±0.25[a]	45.4±3.15[b]	900.6±49.68[a]

Data are presented as mean ± SD. Data were analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns

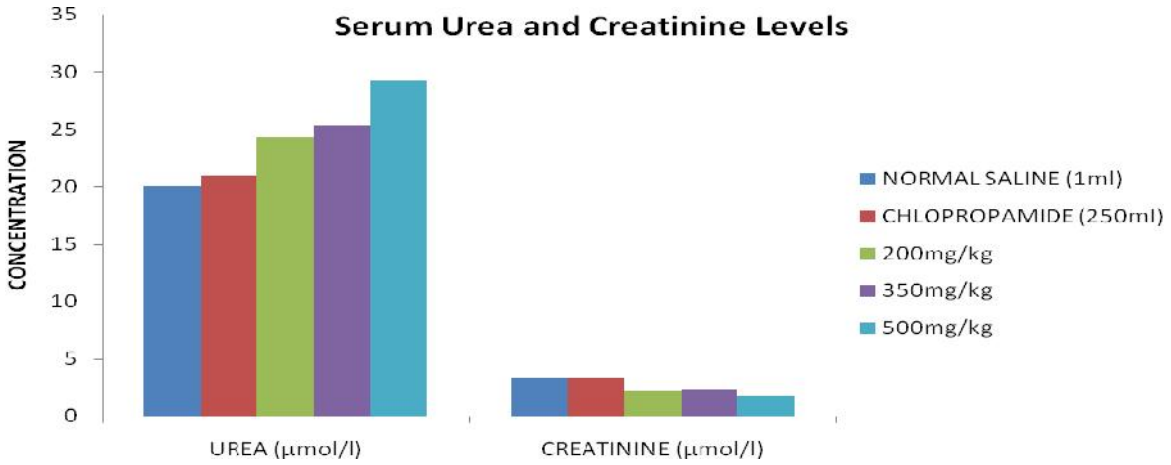

Figure 1: Effect of Aqueous Leaf- Extract of *Desmodium salicifolium* on Serum Urea and Creatinine Levels of Diabetic Rats

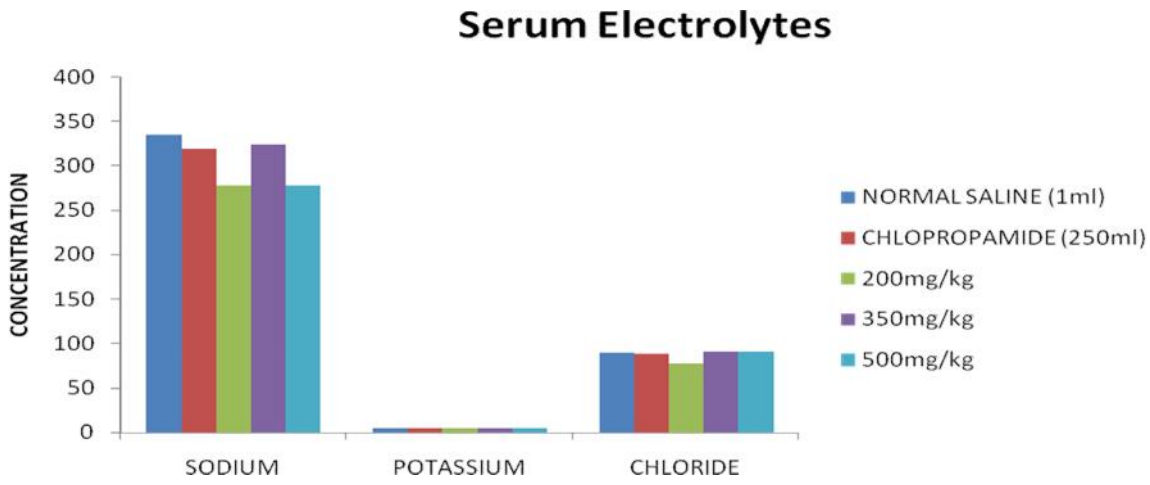

Figure 2: Effects of Aqueous Leaf- Extract of *Desmodium salicifolium* on Serum Electrolyte Levels of Diabetic Rats

4.0. Discussion

Diabetes mellitus is possibly the world's highest metabolic disorder, and as knowledge of its heterogeneity is advancing, the need for more appropriate therapy increases. This disease causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease and heart disease. There is an increase demand to use natural products (herbs) with anti-diabetic activity due to the side effects associated with the use of insulin and oral hypoglycaemic agent.

Haematological changes consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazz *et al.,* 2004). This is presented by alteration in platelet count and activity, coagulopathy, fibronolytic aberration, haemorrhagic factors and changes in endothelial metabolism (McFalarnce, 1997). In this study, diabetic rats were observed to have alterations in hemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) count. The significant (P<0.05) reduction in the hemoglobin, and RBC cell as it pertains to hematology of the diabetic, terms such as anaemia in diabetes, atherosclerosis resulting from increased platelet aggregation, glycosylation of hemoglobin and of recent, even white blood cells have been discussed extensively (Saliu *et al.,* 2012). Table 1 show that there was a significant increase in the WBC count of the diabetic rats. The increased immune cell counts may be the manifestations of the low grade inflammatory reactions associated with the atherosclerotic complications of diabetes mellitus (Hansson, 2005). Platelets have been prominently and critically implicated in the onset and pathogenesis of cardiovascular diseases (CVD) either of diabetics or of other causes. Treatment with the extract showed restoration of the haematological parameters.

Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, and blood clotting and muscle contraction. Electrolyte imbalance resulting from kidney failure has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders (Rao, 1992).The association between blood glucose and serum electrolytes is a complex one and is related to a number of other factors such as age and associated conditions. In this study, there were no significant changes in the levels of serum sodium, pottasium and chloride ions were recorded in the rats following treatment with alloxan and subsequently, the extract. Plasma creatinine and urea levels are established markers of glomerular filtration rate (GFR) (White *et al.,* 1973). They are therefore useful clinical tools in assessing renal function. Creatine is a non-protein nitrogen compound synthesized in the kidney, liver and pancreas from arginine, glycine and methionine (White *et al.,* 1973). After phosphorylation it provides energy for muscular contraction and it is converted into the waste product creatinine (White *et al.,* 1973), which is excreted in urine. Urea is a nitrogenous product of protein metabolism. It is usually excreted from the body via the urine. Creatinine and urea blood concentrations increase in case of any abnormality in kidney function. As their values exceed the normal limit, impairment in kidney function occurs (White *et al.,* 1973). Hence, creatinine and urea clearance is the best measure to check the glomerular filtration and its level in the blood reflects the functional state of the kidney (Munir *et al.,* 2003). Higher creatinine and urea levels may be detected in patients with history of diabetes or heart attack (Salive *et al.,* 1995). The increase in urea levels observed following extract treatment might be as a result of a fall in the filtering capacity of the kidney thus leading to accumulation of these waste products within the system (Idonije *et al.,* 2011).

5.0. Conclusion

In conclusion, the aqueous leaf extract of *Desmodium salicifolium* exhibited beneficial effects on haematological parameters such as red blood cell, white blood cell, haemoglobin concentration, of rats. However, the effect seems not to have a beneficial effect on the kidney as it increased the level of serum urine but decreased serum creatinine levels.

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