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EFFECTS OF AQUEOUS EXTRACTS OF *NAUCLEA LATIFOLIA* ON SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR ALBINO RATS.

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KeyWords

Diabetes mellitus, *Nauclea latifolia*, extracts, albino rats, glibenclamide, biochemical parameters, haematological parameters.

ABSTRACT

Diabetes mellitus is still one of the age-long chronic diseases confronting the human race. Its frontiers are expanding by the day and has become a growing problem in the world today. Sequel to this, robust researches into it with an aim of developing more appropriate drugs for its management have been encouraged in recent times. In this study, the effects of *Nauclea latifolia* extracts on some biochemical and haematological parameters in diabetic rats were investigated. Thirty male Wistar albino rats were divided into six groups of 5 rats each (Groups A – F). Group A (normal control) and Group B (diabetic control) were administered 1 ml distilled water. Groups C, D and E were diabetic rats treated with 500mg / kg. b.wt of extracts of *Nauclea latifolia* while group F received 5mg/ kg. b.wt of glibenclamide. Serum total protein, albumin, urea and creatinine were determined using kits based on standard methods. Haematological parameters were determined using Mindray B C 2800 Veterinary Auto Haematology Analyser. The extracts raised non-significantly total protein and albumin levels while causing non-significant reduction in urea and creatinine. Packed cell volume (PCV), platelets, red blood cells (RBC) and white blood cells (WBC) were significantly increased in the diabetic treated rats. While the extracts do not affect the biochemical parameters significantly, they caused significant increase in the haematological parameters. The significant increase of PCV and RBC by the extracts shows that they have the potential for remedying anaemic condition in diabetes mellitus. The significant increase in platelets by the extracts indicates that they might have beneficial effect in bleeding disorders when used in clinical medicine in addition to having potential to boost immunity since they significantly increased the white blood cells count in the diabetic treated rats.

1. INTRODUCTION

Diabetes mellitus is a major health problem globally. The incidence has remarkably increased in recent times especially in developing and industrialized countries (Haolat *et al*., 2020). Indeed, diabetes mellitus has increased its frontiers due to population growth, urbanization and increasing prevalence of physical inactivity and obesity (Motahareh *et al*., 2021; Etuk, 2010). It is characterized by prolonged unusually high concentration of glucose in the blood as a result of derangement in carbohydrate, protein and fat metabolism (Bamanikar *et al*., 2016; Kanwar *et al*., 2015). The basic mechanism underlying this abnormally high blood glucose involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased use of glucose by the tissues (Maithili *et al*., 2011). Diabetes mellitus results from absolute or relative

deficiencies in insulin secretion, insulin action or both (Kanwar *et al*., 2015).

One of the most significant clinical features of this disease is its associated complications which affect adversely vital organs like the kidney, liver, eyes and the heart. It causes pathological conditions such as nephropathy, hepatopathy, retinopathy and cardiovascular diseases (Ademola *et al*., 2017; Haolat *et al*., 2020). Previous report approximated that 20-30% of all diabetic patients develop evidence of nephropathy with a higher percentage of type 1 diabetic patients progressing to end stage renal disease. Diabetic nephropathy is associated with both macro albuminuria and unusual kidney function as shown by a decrease in glomerular filtration rate and elevated serum urea and creatinine levels (Bamanikar *et al*., 2016). Diabetes mellitus is also associated with haematological disorders (Oyedemi *et al*., 2011; Dasofunjo *et al*., 2013).

Because of its high prevalence and potential harmful effects on a patient's psychological and physical state, diabetes mellitus is a major medical concern. This disease remains incurable and can only be controlled with some drugs which are in themselves associated with some adverse side effects such as interference with renal and hepatic functions, gastrointestinal reactions and coma due to hypoglycaemia (Etuk, 2010; Ochalefu *et al*., 2018). Chemotherapeutic agents have been used for the management of diabetes mellitus since the accidental discovery of the hypoglycaemic action of sulfonamides in 1942. Life style measures and injectable insulin therapy have also been used (Atangwho *et al*., 2007). The essence of these management measures is to achieve an efficient blood glucose control with an aim to delaying or preventing the onset of diabetic complications. These measures are not without limitations and side effects (Atangwho *et al*., 2007). Because of the foregoing, there has been a continuous search for the discovery of plant medicinal agents with novel properties that can be used for the development of anti-diabetic drugs with very minimal side effects in addition to being readily available in the drugs market (Jardin *et al*., 2013; Ochalefu *et al*., 2018).

The plant *Nauclea latifola* being investigated has been shown to contain secondary metabolites which include saponins, flavonoids tannins, alkaloids and phlobatanins (Ochalefu *et al*., 2018). It has a wide usage in folkloric medicine for the management of wounds, cough, gonorrhea, malaria, epilepsy and anxiety (Germain *et al*., 2010; Gidado *et al*., 2012). It is used with some beneficial testimonies among the Idoma natives of North Central Nigeria for the management of diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Reference drug

The anti-diabetic drug used for the management of diabetes mellitus was glibenclamide (Sanofi-Aventis, Nigeria limited) administered orally to the rats at 5mg/kg body weight.

2.2 Plant extracts preparation

Nauclea latifolia parts (stem-bark, leaves and root-bark utilized in this study were harvested from the environment of Joseph Sarwuan Tarka university, Makurdi, Nigeria. Identification and authentication of the plant were done at the Federal School of Forestry, Jos, Nigeria where the voucher specimen with number FHJ 279 was deposited. The stem- bark, leaves and root-bark were dried to constant weights at room temperature for at least four weeks and then pulverized to fine powder with mortar and pestle. This powder was sieved with a porcelain sieve. Then 100 g of the stem-bark, leaves and root-bark powders were

separately macerated in 1000ml distilled water at a ratio of 1: 10 (powder/ solvent) (Das *et al*., 2010).

These mixtures were filtered with muscillin cloth. This was followed with the use of sterile cotton wool and Whatman filter paper No.1 size 110 mm to obtain pure filtrates. The respective filtrates were concentrated on a water bath at 45º C and dried to constant weights. The calculation of the percentage yield was done using the expression:

$$\%\ yield = \frac{Weight\ of\ extract(g)}{Weight\ of\ dry\ sample(g)}\ X\ 10$$

2.3 Induction of Diabetes mellitus

Diabetes mellitus was induced in overnight fasted Wistar rats with single dose of streptozotocin (Sigma-Aldrich, Germany) 60 mg / Kg. body weight administered intra-peritoneally (Adoga and Ibrahim, 1990; Ghoraishian, 2006; Rao and Naidu 2010). Diabetes mellitus was confirmed in the streptozotocin treated rats by measuring fasting blood glucose concentration using glucometer (Accu-Chek, Mannheim, Germany) 48 hours after intra- peritoneal streptozotocin injection. Rats with fasting blood glucose concentration of more than 200 mg / dl were considered diabetic and included in the study.

2.4 Research Design

2.4.1 Animal studies

Thirty male Wistar albino rats weighing 153.5 – 177.0 g used for this study were obtained from the animal house of College of Health Sciences, Benue State University, Makurdi, Nigeria. They were kept to acclimatize for two weeks under normal environmental condition of 12 – hour light and 12- hour dark cycle at room temperature prior to the commencement of the experiment. The rats were grouped into six groups of five rats each. Group A was normal control and group B was diabetic control. These controls were administered 1 ml distilled water orally. Groups C, D and E were diabetic rats treated with 500 mg /Kg. body weight of stem- bark, leaves and root –bark aqueous extracts of *Nauclea latifolia* respectively for 28 days while Group F was diabetic rats treated with 5 mg / Kg. body weight of glibenclamide daily for the same duration (Effiong *et al*., 2013).

The extracts and glibenclamide were administered orally via intra- pharyngeal feeding canula. The rats were fed ad libitum with pellet diet (Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and clean tap water. Proper hygiene was ensured by frequent cleaning and removal of faeces and spills from cages daily. The experiment was conducted between 9. 00 A.M and 11: 00 A.M. The protocols for these experiments were in accordance with the ethical guidelines for the care and use of laboratory animals (NIH, 1985).

2.4.2 Blood collection

Twenty – four hours post treatment with the last dose of the extracts, glibenclamide and the placebo on the test groups and the control respectively, the rats were sacrificed by inhalation of overdose of diethyl ether vapour. Whole blood was collected through retro-orbital sinus. A capillary tube was introduced into the medial canthus of the eye about 30 degrees angle of the nose. A slight thumb pressure was applied to puncture the tissue and to enter the sinus (plexus). The puncturing of sinus led to the gushing out of blood through the capillary tube. After 5 mls of blood had been collected, the capillary tube was gently removed and wiped with sterile cotton wool. The blood for the determination of haematological indices was put into

anticoagulant bottles while the portion for biocehemical parameters determination was put into plain specimen bottles and allowed to stand for 2 hours and then spun with centrifuge (Lemfield Medical England, Model 80-2) at 3000 rpm for 5 minutes. Serum was subsequently harvested from each blood sample with clean Pasteur pipettes and put into clean Bijou bottles until use.

2.4.3 Biochemical analysis

Serum total protein, albumin and urea were determined using Assay kits from Randox Laboratories (Antrim, United Kingdom) based on the methods of Gornal *et al* (1949), Bromcresol Green and Tobacco (1979) respectively, while creatinine level was determined using kits from Teco Diagnostic (Anaheim, USA) based on the method of Hare (1950).

2.4.4 Haematological analysis

Haemoglobin (HB), packed cell volume (PCV), white blood cells (WBC) and red blood cells (RBC) were analyzed using Mindray BC 2800 Veterinary Auto Haematology Analyser

2.5 Statistical analysis

Statistical package for social sciences (SPSS version 24) software package programme was used for the statistical analysis. The data were expressed as Mean ± SEM (standard error of mean) where n=5, and analyzed by One – Way Analysis of Variance (ANOVA). The level of significance was determined by Least Significant Difference (LSD). P values of 0.05 and less were taken to imply statistical significance between the means.

3. Results

3.1 Effect of Stem-bark, leaves and root- bark aqueous extract of *Nauclea latifolia* on some biochemical parameters in streptozotocin-induced diabetic rats.

The serum total protein level in the diabetic control rats reduced significantly ($p < 0.05$) compared with the normal control rats. The administration of the aqueous extracts and glibenclamide slight increase ($p > 0.05$) in total protein levels of the diabetic treated rats. The diabetic control rats had their albumin levels reduced non-significantly ($p > 0.05$) compared with the normal control. The *Nauclea latifolia* extracts caused non- significant increase ($p > 0.05$) in the albumin levels of the diabetic treated rats.

Serum creatinine levels in the diabetic control rats increased non-significantly ($p > 0.05$) compared with the normal control rats whereas the diabetic rats treated with the plant extracts and glibenclamide had their serum creatinine levels reduced non- significantly ($p > 0.05$) compared with that of the diabetic control rats. The diabetic control rats had their urea levels raised non- significantly ($p > 0.05$) compared with the normal control rats. The leaves extract and glibenclamide significantly reduced ($p < 0.05$) serum urea levels in the diabetic treated rats whereas the root – bark and stem – bark extracts causing non- significant reduction ($p > 0.05$) in serum urea levels compared with the diabetic control rats. (Table 1).

Table 1. Effects of stem-bark, leaves and root-bark aqueous extracts of *Nauclea latifolia* on some Biochemical parameters in streptozotocin-induced diabetic rats

Group	Treatment	Total protein (g/dl)	Albumin (g/dl)	Creatinine (mg /dl)	Urea (Mmol/L)
A	Normal control	7.11±0.92	3.63±0.58	1.88±0.26	19.53±1.57
B	Diabetic control	4.98±0.50**	2.80±0.17	2.04±0.36	20.04±1.17
C	Stem-bark extract	5.63±0.41	2.85±0.22	1.80±0.38	18.71±1.58
D	Leaves extract	5.67±2.29	3.40±0.24	1.84±0.21	17.36±1.01*
E	Root-bark extract	5.68±0.54	3.39±0.57	1.74±0.62	19.68±1.83
F	Glibenclamide	5.46±0.57	2.88±0.84	1.94±0.30	18.20±1.15*

Values are Mean ± SEM of 5 determinations.
*= Statistically significant when compared to diabetic control at ($p < 0.05$)
** = Statistically significant when compared to normal control at ($p < 0.05$)

3.2 Haematological Parameters of Wistar Albino Rats.

The packed cell volume (PCV) of the diabetic control rats decreased non-significantly ($p > 0.05$) compared with that of the normal control. The leaves, stem-bark, root-bark extracts caused significant increase ($p < 0.05$) in PCV level compared with the diabetic control. The platelet count was reduced significantly ($p < 0.05$) in the diabetic control rats compared to the normal control. The use of *Nauclea latifolia* aqueous extracts on the diabetic treated rats raised the platelets count significantly ($p < 0.05$) compared to the diabetic control. The red blood cells (RBC) in the diabetic control rats reduced significantly ($p < 0.05$) compared to the normal control. The administration of the extracts to the diabetic treated rats led to significant increase ($p < 0.05$) in their red blood cells level. The white blood cells (WBC) count in the diabetic control rats significantly reduced ($p < 0.05$) compared with that of the normal control. The use of the aqueous extracts and glibenclamide on the diabetic treated rats caused significant increase ($p < 0.05$) in their WBC count compared with the diabetic control rats.

Granulocytes count in the diabetic control reduced significantly ($p<0.05$) compared with the normal control. The administration of the extracts to the treated diabetic rats led to significant rise ($p < 0.05$) in granulocytes level compared with the diabetic untreated rats except for the glibenclamide treated rats where there was significant reduction ($p < 0.05$) in the granulocytes count compared with the diabetic control. Lymphocytes count decreased significantly ($p < 0.05$) in the diabetic control rats compared with normal control rats. The plant extracts and glibenclamide increased significantly ($p < 0.05$) lymphocytes count compared with diabetic control rats. There was no significant difference between monocytes count in the normal control, diabetic control and the diabetic treated with extracts (Table 2).

Table 2. Effects of Aqueous Extracts of Stem-bark, Leaves and Root-bark of *Nauclea latifolia* on Haematological Parameters in Diabetic Wistar Albino Rats.

Group	Treatment	PCV (%)	Platelets ($\times10^9$/L)	RBC ($\times10^{12}$/L)	WBC ($\times10^9$/L)	Granulocytes (%)	Lymphocytes (%)	Monocytes (%)
A	Normal control	44.10±1.08	996.60±2.05	7.81±0.68	9.36±1.86	34.46±5.65	63.86±5.73	3.82±0.15
B	Diabetic control	43.78±1.32	918.40±7.79**	6.32±0.27**	7.84 ±1.64**	32.32±5.83**	40.12±1.15**	3.32±0.23
C	Stem-bark extract	49.54±1.59*	1061.40±1.46*	7.92±1.35*	10.18±3.32*	56.30±1.05*	61.94 ±5.75*	3.53±1.39
D	Leaves extract	48.02±0.41*	1053.40±1.56*	8.18±0.33*	11.30±0.69*	37.82±5.56*	61.76±5.72*	3.98±0.38
E	Root-bark extract	45.36±1.23*	1121.00±2.34*	8.29±0.32*	12.12±3.23*	39.60±7.31*	53.78±7.72*	3.38±0.55
F	Glibenclamide	46.28±2.76*	1015.40±2.27*	8.56±0.49*	10.92±1.76*	29.66±3.20*	66.18±3.19*	3.42±0.54

PCV= Packed cell volume
RBC= Red blood cell
WBC= white blood cell
Values are Mean± SEM of 5 determinations.
*= Statistically significant when compared with diabetic control at ($p < 0.05$)
**= Statistically significant when compared with normal control at ($p < 0.05$)

4. Discussion

Diabetes mellitus is a major cause of kidney disease (Sankeerthi *et al*., 2022). This metabolic disorder contributes greatly to the significant proportion of the burden of kidney damage and dysfunction (Amartey *et al*., 2015). The disease also has adverse effects on haematological parameters (Oyedemi *et al*., 2011).

In this study, serum total protein levels of the diabetic control rats were found to be significantly reduced ($p < 0.05$) compared with the normal control rats. The significant reduction in total protein in the diabetic rats could be that the reduction in glucose disposal and fuel flux in hyperinsulinaemic clamp in type 2 diabetes mellitus might cause a reduction or impairment of mitochondrial adenosine triphosphate (ATP) production which in turn hampers ATP dependent processes like protein synthesis. Thus the reduction in the total protein level in the diabetic rats (Petersen *et al*., 2003; Short *et al*., 2005). The reduction in the total protein level could also be as a result of reduced uptake of amino acids by the tissues and increased rate of muscle proteolysis (Haolat *et al*., 2020; Ahmed 2005). This finding concurs with earlier research work by Shanmugasundaram *et al* (2011) and Ayinla *et al* (2014) who independently reported of diabetes mellitus causing significant reduction in total protein in experimental rats. Nazki *et al*. (2017), however, reported an increased total protein in diabetic mellitus. The administration of *Nauclea latifolia* aqueous extracts and glibenclamide caused slight increase in the total protein levels in the diabetic treated rats. The non-significant increase in total protein level of the extract treated diabetic rats might be that the extract caused inhibition of proteolytic activity due to improved insulin secretion and adequate utilization of blood glucose (Sundaran *et al*., 2009).

Albumin is a small protein that is made in the liver and constitute a major protein in the blood serum. It performs some vital functions which include nourishing tissues, transporting various substances in the body (hormones, vitamins, drugs and ions) and preventing fluid from leaking out of the body vessels. The slight

reduction in serum albumin level in the diabetic control rats compared with the normal control rats might be as a result of diabetes mellitus causing a decreased liver albumin synthesis which is secondary to liver disease. In addition, it could be reduced through a mechanism related to the formation of glycated albumin (Douglas *et al*., 2019). Glycated albumin has unusual ability to bind various ligands and acts as a precursor to advance glycation end-products leading to oxidative stress and inflammation. These processes lead to the clearance of glycated albumin. There is also evidence that glycated albumin cause an immunological response which might further reduce albumin level (Bhat *et al*., 2017; Douglas *et al*., 2019). Abasi *et al* (2012) and Khan *et al* (2010), however, found a significant reduction in albumin level in untreated diabetic state compared with normal control. The slight increase of the albumin level in the extract treated diabetic rats compared with the diabetic control could be that the extracts have some hepatoprotective effect against the over production of reactive oxygen species with its deleterious effect of oxidative stress. The extract might have also led to inhibition of proteolytic activity due to enhanced insulin secretion and proper utilization of blood glucose (Narendhirakannan *et al*., 2006; Sivajothi *et al*., 2007; Sundaran *et al*., 2009).

The diabetic control rats had their creatinine and urea levels increased non- significantly compared with the normal control rats. Bamanikar *et al* (2016) and Shrestha *et al* (2008) in their independent research work found significant increase in both serum creatinine and urea levels in diabetic rats compared with normal control rats. While all the aqueous extracts of the plant non- significantly reduced creatinine levels in the treated rats, the leaves extract and glibenclamide administered diabetic rats showed significantly decreased urea level. The observed decrease in creatinine and urea levels in the diabetic treated rats with the extracts might be as a result of improved renal function secondary to a reduction in blood glucose concentration and its attendant glycosylation of renal basement membrane. Phytochemical screening of the extracts of this plant showed the presence of flavonoid which constitute active biological principle which could have induced the observed effect (Maithili *et al*., 2011; Ochalefu *et al*., 2018).

Serum creatinine and urea levels are usually used as biomarkers for the correlation of the progression of diabetic kidney disease (Sankeerthi *et al*., 2022). Creatinine is a metabolite of muscle creatine whose amount in serum is proportional to the body's muscle mass. The amount of creatinine is usually constant so that elevated levels indicate impaired kidney function since it is easily excreted by the kidneys (Atangwho *et al*., 2007; Haolat *et al*., 2020). Urea is the predominant nitrogen waste product of protein metabolism. As the rate of proteolysis rises in diabetes mellitus, the production of urea by the liver increases. Increase in blood urea level is seen when there is damage to the kidney because urea is usually excreted in the urine (Haolat *et al*., 2020; Shrestha *et al*., 2008; Anjaneyulu *et al*., 2004).

Our results showed that the untreated streptozotocin- induced diabetic rats had decreased red blood cells and packed cell volume thereby creating anaemia in the rats. These findings are in agreement with Shehu-Tijani *et al* (2016) who reported that diabetes mellitus is associated with increased risk of anaemia in rats. The anaemia seen in these streptozotocin –exposed rats could be attributed to increased elimination of erythrocytes. Cell membrane deterioration due to streptozotocin exposure could cause red blood cells haemolysis (Shehu- Tijani *et al*., 2016). In diabetes mellitus the life span of red blood cells may be reduced as a result of disturbances in the haematological milieu, such as chronic hyperglycaemia and hyperosmolarity. These disturbances can result in increased internal viscosity and increased membrane rigidity of these red blood cells which predisposes them to being haemolysed (Rafae *et al*., 2019).

Treatment of the diabetic rats with extracts of *Nauclea latifolia* caused increase in both the packed cell volume and the red blood cells count. This is an indication that the plant extracts may contain some bioactive principles that can stimulate the formation and secretion of erythropoietin in the rats. Erythropoietin is a glycoprotein hormone that stimulates the stem cells in the bone marrow to produce red blood cells (Abu-Zaiton, 2010; Oyedemi *et al*., 2011).

The untreated diabetic rats had their platelets levels significantly reduced in this study. This might be due to platelet aggregation which occurs in diabetes mellitus with long term poor glycaemic control because of deficiency of insulin (Oyedemi *et al*., 2011). Platelets play critical roles in mediating blood clotting, which is a meshwork of fibrin fibres. These fibres adhere to any vascular opening and thus prevent further blood clot. It helps in reducing blood loss and repairing of vascular injury (Oyedemi *et al*., 2010). The plant extracts treated rats had their platelet levels remarkably raised. This effect shows that the plant extracts might have the ability to stimulate the biosynthesis of platelets due to the presence of active compounds that might help to precipitate blood coagulation or clotting particularly during severe haemorrahage (Oyedemi *et al*., 2011).

The significant reduction in the white blood cells (WBC) count in the diabetic untreated rats observed by us might be as a result of the effect of the drug, streptozotocin, that was used for the induction of diabetes mellitus in the rats. Intra- peritoneal injection of streptozotocin is known to suppress the immune system by damaging the WBC count and its differentials such as lymphocytes, granulocytes and monocytes. The decrease of these parameters could be due to suppression of leukocytosis in the bone marrow which may account for poor defensive mechanism against infection. Form the foregoing, they might have effect on the immune system and the phagocytic activity of the rats. (Aboyade *et al*., 2009; Oyedemi *et al*., 2011; Dasofunjo *et al*., 2013).

The aqueous extracts of the plant significantly raised the WBC counts of the diabetic treated rats. The presence of phytochemical like flavonoid which has the ability to stimulate the production of WBC in the extract could be responsible for these observed results in the diabetic treated rats (Johnson *et al*., 2012; Dasofunjo *et al*., 2013).

5. Conclusion

The extracts of *Nauclea latifolia* caused significant increase in packed cell volume, platelets, red blood cells and white blood cells count in diabetic rats. They, therefore, have the potential that might be beneficial in managing anaemic condition, bleeding disorders as well as boosting immunity in clinical medicine.

6. Conflict of interest

The authors declare lack of conflict of interest.

7. Ethical approval

The ethical guidelines for the use and care of research animals were followed.

8. Acknowledgement

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