

# HEMATOLOGICAL PARAMETERS OF ALLOXAN-INDUCED DIABETIC RATS TREATED WITH ETHANOL EXTRACTS AND FRACTIONS OF NAUCLEA LAFILOIA LEAF

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## Abstract

Hematological parameters which have been implicated in diabetes mellitus were investigated in this study. N-hexane, ethyl acetate, butanol and methanol fractions of the ethanolic leaf extract of *Nauclea latifolia* were orally administered once daily for 2 weeks to diabetic rats. The levels of RBC, Hb, HCT, MCV, MCH, MCHC, PLT, PCT, MPV, PDW, WBC, lymphocyte and granulocyte were evaluated in blood. There was significant reduction ( $P < 0.05$ ) in RBC and HCT levels in the treatment groups of ethyl acetate fraction (250 mg/kg) and ethanol extract (250 mg/kg) with significant increases ( $P < 0.05$ ) in their MCV and MCH levels when compared with the diabetic control group. Significant increases ( $P < 0.05$ ) in PLT levels of the treatment groups of ethanol extracts, n-hexane fractions and ethyl acetate fraction (100 mg/kg); PCT levels of ethanol extracts group and MPV levels of ethyl acetate fractions treatment groups was high. The treatment groups of glibenclamide, butanol, methanol, n-hexane, ethyl acetate fractions and ethanol extract (250 mg/kg) showed significant reduction ( $P < 0.05$ ) in their WBC and lymphocyte levels while significant increase ( $P < 0.05$ ) in granulocyte levels was noted in the treatment group of ethanol extract (100 mg/kg) when compared with diabetic control group. In conclusion, the ethanol extract proved to have anti-infective property. Some fractions, showed capabilities to boost the immune system.

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**Keywords:** Hematological parameters, *Nauclea latifolia*, diabetes

## **Introduction**

Diabetes mellitus is an endocrine disorder with different aetiologies, it is characterized by aberration in carbohydrate, protein, blood relating functions and fat metabolism caused by complete or relative insufficiency of insulin secretion and action (Lebovitz, 1994; Andreoli et al., 1990).

Herbal medicine involves the use of herbs and plant parts (roots, stems, leaves, barks, or even fruits to promote and improve health (Akinyemi et al., 2005). Traditional medicinal practices on the African continent dates as far back as 4000 years and were the sole medical systems for health care before the advent of orthodox medicine (Eseyin et al., 2005). Plants are the basis for the development of modern drugs and medicinal plants have been used in many years in daily life to treat diseases all over the world (Agbor et al., 2007).

*Nauclea latifolium* is a struggling shrub called pincushion tree. It is grown in Africa and Asia (Okwori, *et al.*, 2008). In Akwa Ibom State, Nigeria, it is called Mbom-Ibong (Akpanabiatu et al., 2005) while the Northern part of Nigeria name it Tabashiya (Gidado et al., 2008). *Nauclea latifolium* leaves have been reported to have many medicinal potentials like antidiabetic and hypoglycemic property (Gidado et al., 2008; Asanga et al., 2012), hypolipidemic and hypocholesterolemic property (Asanga et al., 2012), anti-hypertensive property (Nworgu et al., 2008). Some plants extracts have been reported to destroy RBC thus leading to anemia (Adedapo et al., 2007). Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts and free radicals from alloxan on the blood constituents of an animal (Mohammed et al., 2009). The aim of this study was to determine the effects of ethanol extract and fractions of *Nauclea latifolium* leaves on the hematological property of alloxan-induced diabetic wistar rats.

## **Materials And Methods**

Fresh leaves of *Nauclea latifolium* were obtained from the endocrine farm of Biochemistry Department, University of Calabar, Cross River State, Nigeria. The leaves were identified in the department of Pharmacognosy, University of Uyo, Akwa Ibom State, Nigeria. They were washed, dried under shade, blended to powder (2kg). The powder was doubly macerated in 20 litres of 95% ethanol, filtered and concentrated in vacuo (40°C). The concentrated ethanol extract (363.07g) was successively partitioned with n-hexane (4 x 250ml), ethyl acetate (3 x 250ml), butanol (4 x 250ml) and methanol (1 x 250ml) and all were concentrated in vacuo to obtain their respective fractions (Venkateswarlu et al., 1993).

## **Animals/Diabetes Induction**

Eighty albino (wistar) rats of weight range (100-250g) obtained from animal house of department of Pharmacology and Toxicology, Faculty of

Pharmacy, University of Uyo, Nigeria, were fed ad libitum with commercial feed and clean drinking water and acclimatized for two weeks before the experiment. They were fasted overnight and injected with alloxan monohydrate (Sigma St. Louis, Mo, USA) intraperitoneally at a dose of 150 mg/kg body weight of rats as 5g/100ml distilled water. After 4 days, seventy surviving rats with blood glucose levels above 250 mg/dL were considered diabetic and used for the study (Katsumata et al., 1999; Dhandapani et al., 2002 ).The experiment was conducted in compliance with ethical guide for care and use of laboratory animals in the University of Uyo.

### **Treatment groups**

The treatment groups used for the study were: Diabetic control groups (30% Tween 80), glibenclamide (5mg/kg), ethanol extract (100 mg/kg), ethanol extract (250 mg/kg) n-hexane fraction (100 mg/kg), n-hexane fraction (250 mg/kg), ethyl acetate (100 mg/kg), ethyl acetate (250 mg/kg), butanol fraction (100 mg/kg), butanol fraction (250 mg/kg), methanol (100 mg/kg) and methanol (250 mg/kg). Each treatment group consisted of five rats and the respective fraction and extract were administered orally once daily via feeding tube for a period of two weeks after which the animals were fasted overnight, anesthetized under chloroform vapour, dissected, their blood collected through cardiac puncture and used for the various hematology assay using the Mind Ray Automated Haematology Analyser, Japan.

### **Statistical Analysis**

The results in are presented as mean  $\pm$  SEM at  $P < 0.05$ . The group data were compared statistically using student t-test and one-way-ANOVA.

## Result And Discussion

**Table 1:** Effect of ethanolic leaf extract and fractions of *Nuclea latifolia* on hematological parameters.

S/N	Treatment groups	WBC X10 <sup>9</sup> /L	Lymph X10 <sup>9</sup> /L	Granulo cytes x10 <sup>9</sup> /L	PLT x10 <sup>9</sup> /L	PCV(%)	MPV (fL)	PDW	RDW C X10 <sup>12</sup> /L	HbG (g/L)	MCV (fL)	MCH (pg)	MCH C (g/L)	Hct (%)
1	Diabetic control (30% Tween 80)	26.3	100.88	1.30	180.40	0.14	8.22	14.74	3.07	83.6	82.3	16.88	239.0	31.74
		± 41.07	± 41.07	± 0.08	± 4.01	± 0.02	± 0.36	± 0.16	± 0.03	± 1.61	± 0.53	± 0.30	± 16.94	± 0.49
2	n-hexane fraction (100mg/kg)	30.80	44.14	28.70	642.00	0.52	9.32	14.92	2.04	96.4	63.98	33.46	1325.0	122
		± 7.65*	± 11.51*	± 16.23	± 137.26*	± 0.08	± 0.48	± 0.44	± 1.20	± 11.53	± 3.76	± 6.80	± 765.72	± 6.71
3	n-hexane fraction (250mg/kg)	34.40	28.73	25.42	673.4	0.82	8.96	14.76	3.11	109.6	33.48	16.88	329.3	322
		± 10.63	± 7.26*	± 5.89	± 54.48**	± 0.01	± 0.47	± 0.33	± 0.64	± 8.70	± 2.01	± 0.41	± 41.90	± 3.70
4	Ethyl acetate fraction (100mg/kg)	64.60	36.46	17.20	564.0	0.38	9.30	14.90	2.79	108.3	57.52	322	571.2	114
		± 5.52*	± 4.13*	± 1.46	± 135.30*	± 0.07	± 0.52	± 0.47	± 1.47	± 21.29	± 0.79	± 5.96	± 95.60	± 8.42
5	Ethyl acetate fraction (250mg/kg)	38.36	21.26	10.38	482.2	0.30	10.70	15.82	0.28	118.3	76.34	380.8	4083.1	2.3
		± 11.80	± 6.17*	± 3.52	± 91.04	± 0.10	± 0.55*	± 0.35	± 0.04	± 14.08	± 2.48b	± 25.20	± 1123.3	± 0.42
6	Butanol fraction (100mg/kg)	25.3	13.2	8.70	401.20	0.33	8.20	14.80	7.12	118.4	37.38	16.88	290.2	40.83
		± 2.67*	± 2.49*	± 1.92	± 42.54	± 0.12	± 0.12	± 0.09	± 0.71	± 10.44	± 1.43	± 0.25	± 3.11	± 3.39
7	Butanol fraction (250mg/kg)	15.20	10.32	5.74	378.00	0.29	8.00	14.24	8.0	138.3	37.38	16.72	286.2	45.14
		± 0.85*	± 0.55*	± 0.68	± 41.87	± 0.03	± 0.08	± 0.18	± 0.11	± 2.95	± 1.02	± 0.33	± 3.17	± 0.89
8	Methanol fraction (100mg/kg)	15.02	10.20	8.10	322.00	0.27	8.30	15.00	6.84	114.0	80.3	17.1	282.2	40.34
		± 2.41*	± 0.54*	± 0.08	± 30.68	± 0.06	± 0.44	± 0.17	± 0.49	± 9.30	± 2.11	± 0.50	± 4.20	± 3.27
9	Methanol fraction (250mg/kg)	15.98	10.30	5.74	387.37	0.30	8.22	14.78	6.14	104.4	80.42	16.9	281.3	38.34
		± 3.56*	± 1.95*	± 0.12	± 6.55	± 0.06	± 0.04	± 0.30	± 0.96	± 16.29	± 2.41	± 0.38	± 6.28	± 5.36
10	Ethanol fraction (100mg/kg)	136.7	31.42	26.72	326.80	0.88	9.38	14.88	2.71	123.3	37.38	204.2	388.8	183.2
		± 35.94	± 16.56	± 14.40*	± 54.40**	± 0.03**	± 0.27	± 0.27	± 0.57	± 24.14	± 5.30	± 4.85	± 23.38	± 2.96
11	Ethanol fraction (250mg/kg)	78.08	31.44*	17.36*	770.30	0.83	9.72	15.42	1.41	115.82	74.76	103.0	1140.3	127
		± 49.12	± 11.04*	± 1.92	± 86.26**	± 0.01**	± 0.60	± 0.27	± 0.48	± 16.70	± 5.01b	± 27.08	± 200.91	± 3.65
12	Ginkgo biloba (5mg/kg)	20.72	12.88	8.38	322.81	0.28	8.30	14.38	8.09	109.2	82.82	17.44	277.0	38.08
		± 4.77*	± 2.83*	± 1.59	± 20.09	± 0.02	± 0.11	± 0.15	± 0.74	± 12.21	± 0.69	± 0.22	± 5.09	± 5.00

\*= significant decrease, \*\*= significant increases at p < 0.05

The results are as presented in Table 1. The primary reasons for assessing the RBC is to check anemia and to evaluate normal erythropoiesis. Hemoglobin level indicates the amount of intracellular iron, while hematocrit, representing the volume of RBC in 100ml of blood helps to

determine the degree of anemia or polycythaemia. The mean cell hemoglobin level is a significant index for folic acid and or Vit B<sub>12</sub> need (Ganong, 1999). The resulting significant reduction ( $P < 0.05$ ) in RBC levels and HCT levels in the treatment groups of ethyl acetate fraction (250 mg/kg) and ethanol extract (250 mg/kg) with significant increases ( $P < 0.05$ ) in their MCV and MCH levels when compared with the diabetic control group may be due to hematotoxic effects associated with toxic substances on bone marrow depression caused by damage to multiple classes of hematopoietic cells and a variety of hematopoietic functions (Synder and Hadli, 1996). Reactive O<sub>2</sub> species generated during alloxan metabolism is implicated in red cell damage (Rao et al., 2003), diabetic rats forms glycosylated hemoglobin hence, decreased total hemoglobin (Sheela and Augusti, 1992). There was no change ( $P < 0.05$ ) in the red cell indices of the diabetic rats treated with butanol, methanol, glibenclamide, n-hexane (250 mg/kg). This result was similar to that reported by some researchers (Mohammed et al., 2009; Edet et al., 2011).

Platelets are fragment of cells that participates in blood clotting, they initiate repair of blood vessels walls and are also considered as an acute phase reactant to infection or inflammation; plateletcrits showcases the precise method of determining the degree of acute blood loss while mean platelet volume (MPV) is used when investigating the ability of a drug to enhance blood clotting (Ganong, 1999). The implication of the significant increases ( $P < 0.05$ ) in PLT levels in the treatment groups of ethanol extracts, n-hexane fractions and ethyl acetate fraction (100 mg/kg) as well as in PCT levels of ethanol extracts groups of rats and MPV levels in the treatment groups of ethyl acetate fractions when compared with diabetic control group is that they are potent as acute phase reactant to infection caused by alloxan free radicals in the diabetic rats. This was consistent with the report (Ajagbonna et al., 1999) on the ability of medicinal compounds or drugs in altering the normal range of hematological parameters. More over, there was no significant change in PLT levels of treatment groups of glibenclamide, methanol, butanol fractions as well as in PCT levels of treatment groups of glibenclamide, n-hexane, ethyl acetate, butanol and methanol fractions when compared with the diabetic control group, suggesting that the plant fractions may not cause thrombosis.

Alloxan diabetogenesis may cause perturbation in the bone marrow stem cells (Edet et al., 2011). The significant reduction ( $P < 0.05$ ) in WBC and Lymphocytes levels of diabetic rats treated with methanol, butanol, n-hexane, ethyl acetate fractions, glibenclamide and ethanol extract (250 mg/kg) when compared with the diabetic control group gave credence to the abilities of the above treatment groups in curtailing hematological abuses in the defense system of the diabetic rats. The result was not similar to that

reported by some researchers (Mohammed et al., 2009; Akah et al., 2009; Tanko et al., 2011). Also, the possible significant increase ( $P < 0.05$ ) in granulocytes levels of the rats treated with 100mg/kg of ethanol extract when compared with the diabetic control group may indicate anti-infective effect of the extract and this report was similar to the one by (Mohammed et al., 2009; Tanko et al., 2011). More over, there was no change in granulocyte levels noted for the treatment groups of methanol, n-hexane, butanol, ethyl acetate fractions, glibenclamide and 250 mg/kg of ethanol extract. This suggests their abilities in arresting allergy and bacterial infection resulting from free radicals generated by alloxan metabolites on the different tissues of the diabetic rats.

In conclusion, the ethanol extract proved to have anti-infective property, glibenclamide, butanol, methanol, n-hexane, ethyl acetate fractions and ethanol extract (250 mg/kg) showed capabilities to boost the immune system and curtail some hematological abuse in the defense system, while dose dependent ethanol extract and ethyl acetate fractions may reduce erythropoiesis. Also, ethanol extracts, n-hexane fractions and ethyl acetate fraction (100 mg/kg) may act as acute phase reactant to infections associated with pathophysiology of diabetes mellitus.

It is hereby recommended that more work should be done on the ethyl acetate, butanol and methanol fractions to isolate and identify the components responsible for their hematoprotective property.

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