

Evaluation of the ameliorative impacts of ethanol rhizomes extract of *Curcuma longa* on hematological parameters of diabetes- induced male Albino Wistar rats

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Abstract

The ameliorative effects of ethanol rhizome extract from *Curcuma longa* on the haematological indicators of male albino Wistar rats (198-225g) induced by alloxan diabetes were assessed. Thirty healthy animals were randomly divided into six groups of five rats each. Diabetes mellitus was generated in groups 1 to 5 through the peritoneum. Subsequently, groups 1, 2, and 3 received daily oral administration of 200 mg/kg, 500 mg/kg, and 700 mg/kg of *C. longa* extract, respectively, for one month. Group 4 received 500mg/kg of metformin, a typical anti-diabetes medication (positive control), whereas groups 5 and 6 were untreated, serving as negative and normal controls, respectively. The haematological indices measured included Red Blood Cells (RBCs), White Blood Cells (WBCs), Platelets (PT), Haemoglobin (Hb), Haematocrits (HCT), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Lymphocytes (LYM), Monocytes (MON), Basophils (BAS), and Eosinophils (EOS). When compared to groups 1, 2, 3, 5, and 6, group 4 had a statistically significant increase ($P<0.05$) in WBCs and PT. Compared to groups 1, 2, 5, and 6, group 4 saw a substantial drop ($P<0.05$) in RBCs and Hb. When compared to groups 2, 3, and 4, group 1 saw a big rise in HCT ($P<0.05$). When compared to groups 3, 5, and 6, group 4 was determined to be significantly lower ($P<0.05$). There was no significant difference ($P>0.05$) between and among groups for MCV and MCH. There was a big difference in MCHC between group 2 and groups 1 and 3 ($P<0.05$). In group 1, lymphocytes dropped a lot ($P<0.05$) compared to groups 2, 3, 5, and 6. When group 4 was compared to group 2, MON went up a lot ($P<0.05$). When compared to groups 4 and 6, group 3 saw a big rise in BAS ($P<0.05$). In group 3, EOS went raised a lot ($P<0.05$) compared to groups 4 and 5. These results suggested that diabetes affected the haematological indices in the mice, while ethanol rhizome extract appears to mitigate the consequences.

Keywords: *Curcuma longa*; Ameliorative Impacts; Hematological; Diabetes Mellitus; Albino Wistar Rats

1. Introduction

Plant materials are essential to both traditional herbal therapy and modern pharmacology because they include a wide range of bioactive substances called phytochemicals. They can cure and manage a wide range of diseases and ailments in many different ways since they have so many different chemicals. Plant extracts may help people with diabetes by controlling their blood sugar levels in a number of ways, such as by raising insulin production, making insulin more effective, lowering glucose absorption, and having antioxidant and anti-inflammatory effects. These extracts contain bioactive chemicals such as flavonoids, alkaloids, and phenolics that interact with metabolic pathways like PIBK/AKt, AMPK, and NF-KB to lower high blood sugar and associated effects. *Curcuma longa* rhizomes, commonly referred to as turmeric, contain the active compound curcumin, which may assist in the management of diabetes by lowering blood glucose levels, enhancing insulin sensitivity, and mitigating comorbidities such as obesity and hypercholesterolaemia (Annapurna et al., 2001).

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Studies indicate that it accomplishes this by blocking enzymes that facilitate sugar metabolism, functioning as an antioxidant, and mitigating inflammation. Diabetes can cause major problems, especially with the heart, kidneys, eyes, nerves, and feet. Heart attacks, strokes, renal damage that can lead to kidney failure, vision loss and blindness, nerve damage that causes pain or loss of sensitivity, and foot problems that can cause ulcers and amputation are some of the most serious effects (Jarald et al., 2008).

Diabetes impacts haematological parameters by modifying the function and form of Red Blood Cells (RBCs), White Blood Cells (WBCs), and platelets (PT), frequently resulting in anaemia, increased WBC and platelet numbers, and alterations in coagulation factors (Akinloye et al., 2014). High blood sugar leads to oxidative stress and advanced glycation end products (AGES), which can cause problems with the endothelium, inflammation, and a higher chance of heart problems.

However, herbal medicines are sometimes preferred because they are cheaper, easier to get, and thought to be safer because they have fewer and less severe side effects than orthodox drugs. There are also documented shortages and access issues, especially for newer, highly effective medications in low- and middle-income countries, which are made worse by manufacturing problems and unprecedented demand. This research aimed to find out how *C. longa* rhizomes extract can improve the haematological indices of male albino Wistar rats with diabetes.

2. Materials and methods

2.1. Collection and Preparation of Plant Sample

A taxonomist from the Department of Botany and Ecological Studies at the University of Uyo in Akwa Ibom State, Nigeria, confirmed that the newly obtained rhizomes of the *Curcuma longa* plant were real (Essien et al., 2022). The rhizomes were peeled with a knife to get rid of the scales, washed with distilled water to get rid of dirt, cut into small pieces with a knife, and dried at room temperature for three days. Then, they were ground with a clean, dry mortar and pestle. Finally, 1000g of the sample was soaked in 200ml of 70% ethanol for 72 hours at room temperature. We used a funnel and Whatman No. 1 filter paper to filter the macerated rhizomes extract. The filtrate was concentrated for three days in a water bath. After that, the extract was turned into a slurry and stored in a refrigerator at 4°C for later usage (Essien et al., 2022).

2.2. Procurement of Alloxan and Induction of Diabetes Mellitus

We got alloxan from a drug business in London and used it in our experiment. In groups 1 to 5, based on the average body weight of the group, 180 mg/kg of alloxan was injected into the peritoneum of albino Wistar rats that had been fasting overnight. After three days of induction, a glucometer and strips proved that the person had diabetes.

2.3. Experimental Design, Grouping and Treatment of the Animals

We got thirty (30) healthy adult male albino Wistar rats that weighed between 198 and 225 grammes from the disease-free stock of the animal house at the Biochemistry Unit, Department of Chemical Sciences, Akwa Ibom State Polytechnic, Ikot Osurua. The animals lived in a cage with big compartments that had a hardwood bottom and a mesh top. They were randomly put into six groups, with five animals in each group. The rats were kept in the animal house at Akwa Ibom State Polytechnic, Ikot Osurua, for 7 days to get used to the temperature and natural light-dark cycle. The utilisation of animals for the research was conducted in compliance with the protocols established by the Institute for Laboratory Animal Research, 2000. Groups 1 to 5 were made diabetic, and then groups 1 to 3 were given 200mg/kg, 500mg/kg, and 700mg/kg of *Curcuma longa* rhizomes extract every day for 30 days. Group 4 was given 500mg/kg of metformin, which is a common anti-diabetes drug (positive control). Groups 5 and 6 were not given any treatment and served as negative and normal controls, respectively. Whole blood was obtained through cardiac puncture while the animals were anaesthetised with chloroform gas and subsequently euthanised via medioventral dissection. We took blood from each rat and tested it for White Blood Cells (WBC), Red Blood Cells (RBC), Platelets (PT), Haemoglobin (Hb), Haematocrits (HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Lymphocytes (LYM), Monocytes (MON), Basophils (BAS), and Eosinophils (EOS).

3. Methods

3.1. Determination of Hematological Parameters

We used a Sysmex Haematological analyser, which is a three-in-one machine that can test 19 different things per sample, to do a complete blood count (FBC). The samples were calibrated according to what the manufacturer said to do. We

put each blood sample into the automated analyser and hit the start button. After 30 seconds, the analysis results were shown, and the analyser printed them down on paper.

2.4.2 Statistical Analysis
Data obtained from the test was subjected to One-Way Analysis of Variance (ANOVA). Statistical significance of differences was obtained at ($P<0.05$) by Benferoni multiple range test. The results expressed at mean standard error of mean (SEM) estimated using Statistical Package of Social Sciences (SPSS) version 26.

4. Results

Table 1 Mean Hematological Indices of male albino Wistar rats Induced with Diabetes and Treated with *Curcuma longa* Ethanol Rhizomes Extract

Hematological parameters	Groups					
	Treated with 200mg/kg 1	Treated with 500mg/kg 2	Treated with 700mg/kg 3	Treated with 500mg/kg of Metformin 4	Treated with Negative Control 5	Not induced nor Treated Normal Control 6
WBCs (m/mm^3)	8.96 \pm 1.40	6.41 \pm 1.10	9.38 \pm 0.72	13.97 \pm 1.77	8.27 \pm 1.30	11.42 \pm 1.65
RBC (m/mm^3)	7.35 \pm 0.25	6.44 \pm 0.31	6.70 \pm 0.30	5.42 \pm 0.42	6.79 \pm 0.20	6.80 \pm 0.15
PT (%)	0.49 \pm 0.03	0.43 \pm 0.05	0.54 \pm 0.08	0.76 \pm 0.08	0.84 \pm 0.03	0.74 \pm 0.04
Hb (g/dl)	14.52 \pm 0.38	13.28 \pm 0.58	13.14 \pm 0.79	11.24 \pm 1.21	13.80 \pm 0.37	13.84 \pm 0.34
HCT (%)	38.75 \pm 1.32	32.14 \pm 1.49	34.02 \pm 1.55	28.14 \pm 2.16	35.10 \pm 1.01	35.54 \pm 0.40
MCH (Pg)	19.76 \pm 0.59	20.75 \pm 0.17	19.52 \pm 0.41	20.64 \pm 1.43	20.26 \pm 0.17	20.30 \pm 0.21
LYM (%)	64.56 \pm 1.41	76.48 \pm 1.56	75.12 \pm 2.68	61.78 \pm 2.57	77.06 \pm 2.58	76.16 \pm 3.54
EOS (%)	4.16 \pm 1.52	3.48 \pm 0.92	6.42 \pm 2.51	1.96 \pm 0.78	2.12 \pm 0.37	4.50 \pm 1.33
MCV (fl)	52.84 \pm 1.00	50.06 \pm 0.65	52.48 \pm 1.68	52.48 \pm 1.68	51.74 \pm 0.60	52.38 \pm 0.84
MCHC (g/dl)	37.48 \pm 0.69	41.32 \pm 0.76	38.46 \pm 0.71	39.62 \pm 1.88	39.28 \pm 0.27	38.90 \pm 0.74
MON (%)	13.44 \pm 0.73	10.18 \pm 0.92	12.18 \pm 2.02	15.82 \pm 1.56	10.92 \pm 1.56	13.00 \pm 1.99
BAS (%)	0.26 \pm 0.05	0.32 \pm 0.04	0.40 \pm 0.07	0.16 \pm 0.09	0.26 \pm 0.51	0.15 \pm 0.62

5. Discussion

Haematology is the study of blood in health and disease. This include problems with the red blood cells (RBCs), White Blood Cells (WBCs), platelets (PT), blood vessels, bone marrow, lymph nodes, spleen and the proteins involved in bleeding and clotting (homeostasis and thrombosis) (Toga, 2007). Haematological disease is a disorder which primarily affects the blood and blood forming organs and this include; anemia, leukemia, lymphoma, platelet disorder, HIV, genetic disorder etc. (Wangh et al., 2001; Bamishaiye et al., 2009). Haematological parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005). Alterations in haematological indices may be caused by diabetes mellitus, infections, Cancer, environmental and occupational exposure, chronic diseases, Pregnancy and nutritional deficiencies (Adrover et al., 2016).

Meanwhile, this study evaluated ameliorative impacts of ethanol rhizomes extract of *Curcuma longa* on haematological parameters of alloxan-induced diabetic albino Wistar rats. Red Blood Cells (RBCs), White Blood Cells (WBCs), Platelets (PT), Haemoglobin (Hb), Haematocrits (HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Lymphocytes (LYM), Monocytes (MON), Basophils (BAS) and Eosinophils (EOS) were the haematological parameters determined. WBCs and PT counts recorded statistical significant increase in group 4 animals treated with 500mg/kg Metformin, a standard anti-diabetic drug, when compared to groups 1, 2, 3, 5 and 6 respectively. Highest level of WBCs ($13.97m/mm^3$) was found in group 4 while the lowest was found in group 2 ($6.4m/mm^3$) which was treated with 500mg/kg of *C. longa* ethanol rhizomes extract. According to Ayoola et al., (2016) and Akinloye et al., (2014), diabetes often leads to an elevated WBCs counts as

observed in this work. The elevated WBCs potentially indicate inflammation and increasing risk of complications though the exact relationship is complex. While high WBCs levels are associated with diabetes, excess blood sugar can also directly hinder WBCs function, impairing the immune system ability to fight infections (Adeyemi et al., 2009). Again, elevated WBCs counts in diabetes are often linked to chronic inflammation, which plays a significant role in the development of diabetes complications. According to Ezeigbo et al. (2021), high WBC counts, particularly neutrophils and lymphocytes, are considered an indicator of increased risk for developing cardiovascular diseases and other serious complications in people with diabetes.

Platelets (PT) also showed a significant increase in group 4 when compared to groups 1, 2, 3, 5, and 6 respectively. The highest PT counts (0.84%) was in group 5 (negative control), while the lowest (0.43%) was in group 2 treated with 500mg/kg *C. longa* rhizomes ethanol extracts. Meanwhile, diabetes has been reported to cause platelets to become hyperactive, leading to increased adhesion and aggregations, which contributes to a thrombotic complication (Sikerwar et al., 2009). This platelet hyper-reactivity is driven by factors like hyperglycemia (causing protein glycation and oxidative stress), insulin resistance (reducing the inhibitory effects of insulin on platelets), and increased mean platelets volume (MPV) (Akinloye et al., 2014; Ayoola et al., 2016). These changes in platelet function can be detected through easily measuring platelet indices and are associated with a higher risk of vascular complications. Furthermore, the RBCs and Hb recorded significant decrease in group 4 when compared to groups 1, 2, 5 and 6 respectively. The highest RBCs (7.35m/mm³) and Hb (14.52g/dl) were found in group 1 animals treated with 200mg/kg *C. Longa* ethanol rhizomes extract whereas the lowest RBCs (5.42m/mm³) and Hb (11.24g/dl) were found in group 4 animals. These results showed that the lowest dosage of the plant extract exhibited the highest boost of RBCs and Hb in the animals and that metformin, a standard antidiabetic drug might have reduced RBCs and Hb in the animals. Moreso, diabetes has been reported to have negatively affected RBCs by increasing glycation and oxidative stress, leading to increased membrane rigidity and decreased deformability (Hassan et al., 2018; Okafor et al., 2019). The hardening reduces RBC flexibility, hindering their ability to pass through narrow capillaries and impairing blood flow to tissues. Consequently, RBCs have a shortened lifespan, contributing to anemia and complications like eye, nerve and kidney damage. High blood sugar levels cause glucose to attach to RBCs and their proteins, a process called glycation. This process which can be measured in the HbA/c test, leads to a "Candy Coating" effect that hardens the cells. Chronic hyperglycemia generates reactive oxygen species (ROS), leading to increased oxidative stress and lipid peroxidation in the RBC membrane, increasing membrane stiffness and reducing its flexibility and fluidity, but, in this study, it is observed that ethanol rhizomes extract of *C. longa* at the lowest dosage (200mg/kg) have boosted RBCs and Hb in the animals.

Haematocrits (HCT) showed significant increase in group 1 when compared to groups 2, 3 and 4. Group 4 was found to be significantly decreased when compared to groups 3, 5, and 6 respectively. The highest HCT (38.75%) was in group 1, while the lowest (28.14%) in group 4 were below the normal range (42-50%) for human (Ezeigbo et al., 2021). Diabetes can impact HCT, by both increasing and decreasing it. High HCT is linked to poorer glycemic control and insulin resistance, while poor control and complication can lower HCT. Higher blood viscosity from elevated HCT can worsen microcirculation, leading to slowed circulation in small vessels and potential damage. However, improved diabetes management can lead to higher HCT levels, reflecting better overall health and reduced microvascular damage.

There was no significant difference between and within groups for MCV and MCH. The highest MCV and MCH in this study were 52.84fl and 20.75pg respectively which were in groups 1 and 2 respectively. Whereas the lowest were, MCV (50.06fl) and MCH (20.26pg) found in groups 2 and 5 respectively. The MCV and MCH between and within groups were below the normal ranges of 80-100fl and 27- 31Pg respectively for humans. Diabetes, particularly, hyperglycemia, can increase MCV due to increased RBC swelling and can also elevate RBC count (Akinloye et al., 2014). Studies have revealed conflicting results, with some finding higher MCV in diabetics, possibly linked to hyperglycemia, while others show less change or even lower MCV as recorded in this work. Elevated MCV is a potential indicator of diabetic complications like neuropathy and macrovascular issue, suggesting it could serve as a predictive marker, for morbidity and mortality in certain diabetes populations. Again, diabetes, particularly hyperglycemia can increase MCH in RBCs. This effect is associated with higher blood glucose levels, which may lead to other changes in red blood cell count and volume (Chaudhary and Tyagh, 2018).

Mean Corpuscular Haemoglobin Concentration (MCHC) showed a significant increase in group 2 when compared to groups 1 and 3. The highest (41.32g/dl) and lowest (37.48g/dl) were found in groups 2 and 1 respectively. Meanwhile MCHC in all the groups of animals in this work were above the normal range (32-36 g/dl) for human. According to Nagappa et al. (2003) and Sabu and Kuttan (2004), MCHC can be either decrease, increase, or show no significant change compared to healthy individuals in diabetic patients, this, the authors suggested that the funding depend on the patient's glycemic control and other diabetes-related factors. However, in this study, diabetic conditions of the animals have made MCHC to be above its normal range for human.

Lymphocytes (LYM) decreased significantly in group 1 when compared to groups 2, 3, 5 and 6 respectively. The highest LYM level(77.06%) was in group 5, whereas, the lowest level (61.78g/dl) was in group 4. Diabetes can impair lymphatic function by damaging the autonomic nerves controlling lymph vessels, leading to lymphedema (swelling), potentially leading to issues with particle uptake, lymphocyte output, and increased susceptibility to infection (Tenpe and Teole, 2009; Tailang et al., 2008). However, in this study, *C. longa* extract at different dosage seems to have regulated the lymphocytes level in the extract treated groups.

Monocytes (MON) significantly increased in group 4 when compared to group 2. Basophils (BAS)recorded a significant increase in group 3, when compared to groups 4 and 6 respectively. The highest MON level (15.82%) was in group 4 while the lowest (10.18%) was in group 2. MON level in all groups of animals in this work were greater than the normal range (2-8%) for human. Again, BAS level was highest (0.32%) in group 2 and lowest (0.15%) in group 6. These fell within the normal range (0-1%) for human. Meanwhile diabetes profoundly affects monocytes, a type of WBC, by altering their number, function and behaviour, which contribute significantly to inflammation and the development of complications, particularly cardiovascular disease (Hassan et al., 2018; Ezeigboetal., 2021).

The chronic high blood glucose (hyperglycemia) associated with diabetes triggers these changes, making monocytes, more pro-inflammatory add less effective at resolving inflammation. However, diabetes also has a significant effect on BAS, which are a type of WBC involved in immune responses. Research indicates that high glucose levels and chronic inflammation in diabetes can both increase basophil activity and alter their overall count, suggesting that BAS plays a role in the progression and development of diabetes complications. Again, diabetes significantly affects eosinophils, a type of WBC, by altering their levels and function, which contribute to chronic inflammation and can impact disease progression. The specific effects differ between type 1 and type 2 diabetes and involved complex interaction with glucose, insulin, and the immune system. Meanwhile, EOS witnessed a significant increase in group 3 when compared to groups 4 and 5 respectively. The highest value (6.42%) was found in group 3, while the lowest (1.96%) was found in group 4. However, the plant extract used in this work seems to have boosted the WBC components when compared to the positive, negative and normal control groups of animals, making *C.longa* extract to be seen as immune booster in the animals.

6. Conclusion

Ameliorative impacts of ethanol rhizomes extract of *Curcuma longa* on some hematological indices of alloxan induced diabetes, albino Wistar rats were assayed. It was established that diabetes mellitus impacted negatively on the hematological parameters studied, however, ethanol rhizomes extract exhibited restorative effects on the hematological indices.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed

Statement of ethical approval

There are no ethical conflicts existing with the use of animal in this work.

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