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Effect of Therapeutic Properties of *Curcuma Longa* Rhizomes Extract on Some Selected Haematological Indices of Male Albino Wistar Rats Induced with Diabetes Mellitus

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Abstract: *Curcuma longa* (Turmeric) is a nutraceutical plant commonly used as dietary spice in many cuisines, as analgesic agent to relief pain and traditionally in the treatment of various ailments. This study investigated the therapeutic effect of *Curcuma longa* rhizomes extract on some selected hematological indices of male albino Wistar rats induced with diabetes mellitus. Thirty (30) healthy and sexually matured male rats weighing between 180-220g were randomly distributed into six groups of five rats in each group, viz: group one served as Diabetic induced (180mg/kg alloxan prepared) + 200mg/kg (rhizomes extract of *curcuma longa*), group two as Diabetic induced (180mg/kg alloxan prepared) + 500mg/kg (rhizomes extract of *curcuma longa*), group three as Diabetic induced (180mg/kg alloxan prepared) + 700mg/kg (rhizomes extract of *curcuma longa*), group four as Diabetic induced + 500mg/kg of metformin, group five as Diabetic induced (negative untreated) and group six as Normal Control. At the end of the experiment, Neutrophil (Neu), Red Cell Distribution Width (RDW), Thickness Ratio of the Red Blood Cell (THR) and Mean Platelet Volume (MPV) were evaluated. Results indicated that significant ($P < 0.005$) increase in the five hematological indices levels across the induced diabetic groups as compare to normal control (uninduced) group. However, the treated diabetic groups such as Diabetic induced + 200mg/kg (rhizomes extract of *curcuma longa*), Diabetic induced + 500mg/kg (rhizomes extract of *curcuma longa*), and Diabetic induced + 700mg/kg (rhizomes extract of *curcuma longa*) respectively indicated significant ($P < 0.005$) decrease in the level of the hematological indices use in this study compare to diabetic induced untreated group. Also, the induced diabetic group treated with standard anti-diabetic drug (metformin) shewed significant ($P < 0.005$) decrease in the levels NEU, RDW, THR and MPV as compare to untreated diabetic group. Furthermore, both rhizomes extract of *curcuma longa* and metformin reduces the elevated hematological parameters level in this study to a comparable level with normal control (uninduced) group. It can be concluded that the administration of graded doses (200mg/kg, 500mg/kg and 700mg/kg) of rhizomes extract of *curcuma longa* to diabetes induced group in the present study is effective in the management of hematological disorder that may arise from diabetes mellitus complications.

Keywords: Turmeric, Neu, MPV, RDW, THR, Diabetes Mellitus, Animal Model (Albino Wistar Rats)

Introduction

The use of medicinal plant like *Curcuma longa* in ethnomedicinal practices are a core component in modern drug discovery and development, this is fundamentally due to its availability, affordability, and efficacy with minimal side effect depending on the doses upon the administration of the plant sample. Turmeric (*Curcuma longa*) is a nutraceutical and edible perennial rhizome plant of *Zingiberaceae* (Ginger) family. The plant is known for its rich bioactive constituents which is widely used in diet as spices, as an analgesic agent, in the treatment of wounds and diseases [1]; Braga [2]. The plant is extensively reported to have some therapeutic potentials like anti-glyceamic, antioxidant, anti-inflammatory as well as anticancer properties. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin insensitivity, or both.

This condition is associated with several complications, including alterations in hematological parameters which may give rise to severe health condition such as anemia, leukocytosis, change in blood cell morphology and functions. The hematological modification contributes significantly to the illness and mortality observed in diabetic patients. Conventional antidiabetic drugs, though effective in controlling blood glucose levels, often have limited effects on hematological complications and may produce antagonistic side effects with the prolong usage. Therefore, this study seeks to evaluate the therapeutic influence of *Curcuma longa* rhizome extract on some selected hematological parameters in male albino wistar rats induced with diabetes, in order to determine its potential role in easing diabetes associated haematological abnormalities.

Materials and Methods

Collection of Plant and Extraction

The plant sample was collected at Akwa Ibom State polytechnic botanical garden and was authenticated by a taxonomist in the Herbarium Unit, Department of Botany and Ecological Studies, university of Uyo. The plant sample (rhizomes) were peeled with knife, washed with distilled water to remove debris, cut into small sizes with a knife, and thereafter sample were oven dried at 39°C under a control condition for four hours to prevent Charring [3]. The dried sample was pulverized into a powder form with the aid of electric blender to obtain 1300g before extraction. Grounded sample weigh 1300g was soaked in 200ml of 70% ethanol for three days at room temperature. Funnel and Whatman No. 1 filter paper was used to filter the ethanolic macerated rhizomes solution. The filtrate was concentrated for three days in a water bath to obtain a gel-like extract, and was stored in a refrigerator at 4°C for future use.

Experimental Design/ Induction of Diabetes Mellitus /Treatment with plant Extract.

Thirty healthy male albino Wistar rats weighing 180 – 220g were obtained from a disease-free stock of animal's house of Biochemistry Unit, Department of Chemical Sciences, Akwa Ibom State Polytechnic were used for this study. The rats were allowed to acclimatize to handling in the experimental section of the Biochemistry Unit, Akwa Ibom State Polytechnic, Ikot Osurua, Animal House before commencement of experiments. Following the acclimatization, the rats were housed in conventional standard wooden cages with top mesh wire covers under standard laboratory conditions (relative humidity 45%, room temperature of 25°C) and were exposed to twelve hours light and dark cycle with adequate ventilation. They were allowed access to water and pellets feed (rat chow) *ad libitum*. The study was conducted in accordance with the recommendations of the Polytechnic's Ethical Committee on the Care and Use of Experimental Animals. Out of thirty (30) rats used in this study, twenty-five (25) rats were induced with diabetic by injecting 180 mg/kg of alloxan at the peritoneum region of albino wistar rats after twelve hours fast, and induction carried out depend on the average body weight of each group. While group 6 of five (5) rats were uninduced

and serve as a normal control group. After three days of induction, rats from one of the diabetic induced groups were sacrificed, serum collected for diabetic examination with the aid of glucometer and test strips as a biochemical confirmation of successful diabetes induction. Following the confirmation of diabetic state in experimental animals used in this study, group1 (induced diabetes) were treated with ethanol 200mg/kg rhizomes extract of *curcuma longa*, group 2 (induced diabetes) were treated with 500mg/kg rhizomes extract of *curcuma longa*, group 3 were induced with diabetes received 700mg/kg rhizomes extract of *curcuma longa*, group 4 treated were induced with diabetes received 500mg/kg of metformin (standard anti-diabetic drug), while group 5 serve as diabetes induced control (Diabetes -induced without treatment) and group 6 were not induced and serve as negative and normal controls respectively. The standard drug and extracts administration was by oral gavages for four (4) weeks, after first three days diabetes induction period.

Collection of Blood Sample and Preservation of Serum

After thirty (31) days period of feeding, diabetes induction and extract administration, the animals were subjected to overnight fast before sacrifice. In accordance with the standard guidelines, they were anaesthetized under chloroform vapor and were scarified with their thorax opened, and whole blood collected via cardiac puncture by means of 5ml sterile syringes and needles into a well-labeled EDTA- sample bottles for assay of hematological indices. Also, the latter blood samples were allowed to stand for an hour to clot, after which they were centrifuged with MSE model at 4,000 rpm for 25 minutes to obtain serum and later stored in a refrigerator for analysis.

Determination of Haematological Parameters

Blood analysis was performed using sysmex haematological analyzer, a three -in- one automated analyzer machine that can test for the following paramaters: NEU, MPV, THR, RDW; calibrations of the machine were done according to the manufacturer's instruction. Each of the blood samples were inserted into the automated analyzer and the start button was pressed down-ward for reading. The results of the analysis were displayed on the screen after 30 seconds and the analyzer generated a paper copy of the results on the printing paper. 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 as mean standard error mean (SEM) estimated using statistical package of social sciences (SPSS) version 26.

Results

Table 1. Therapeutic Influence of Curcuma longa Rhizomes Extract on Some Haematological Indices in Male Albino Wistar Rats Induced with Diabetes Mellitus.

Hematological Parameters	Groups					
	Treated with 200mg/kg of <i>Curcuma longa</i> ethanolic rhizomes extracts (Group 1)	Treated With 500mg/kg of <i>Curcuma longa</i> ethanolic rhizomes extracts (Group 2)	Treated With 700mg/kg of <i>Curcuma longa</i> ethanolic rhizomes extracts (Group 3)	Treated With 500mg/kg of metformin (Positive control) (Group 4)	Diabetic Induced (negative Control) (Group 5)	Normal control (Group 6)
MPV (FL)	8.56±0.05	8.52 ±0.10	8.30 ±0.19	9.0 2±0.37	13.14 ± 0.21	8.26 ±0.15
RDW (%)	13.47 ± 0.44	13.40 ±0.22	13.36 ±0.52	13.28 ± 0.35	15.10 ± 0.53	12.90 ±0.33
NEU (%)	10.58 ±2.13	8.66 ±1.55	7.88±1.68	8.28 ±3.86	18.28 ±	6.14±0.69

					2.08	
THR (m/mm ²)	790.00 ± 42.93	773.60 ± 60.61	760.00 ± 108.62	793.00 ± 85.25	903.80 ± 24.32	750.80 ± 54.94

Values are expressed as Mean ± SEM, n = 5

MPV - Mean Platelet Volume (fL)

RDW - Red Cell Distribution Width (%)

NEU - Neutrophil (%)

THR - Thickness Ratio of the Red Blood Cell (m/mm³)

This research work was carried out to evaluate the effect of *Curcuma longa* leaf extract on hematological parameters of induced diabetes in male wistar rat models following the interaction of *Curcuma longa* extract with induced diabetic haematological indices. The results indicated reduction of mean platelets volume in the groups treated with the plant extract which compete favourably with the group treated with conventional anti-diabetic drugs. In the same vein, the impact of the extract on rat red blood cell distribution width in induced diabetic rats enhances the distribution volume of red blood cell, hence, reduced the risk of health-related conditions such as hemolytic anemia, bone marrow defects, cardiovascular complication and inflammation that may accompany deficiency or lack of red blood cell distribution. Furthermore, the investigations showed that the plant extract on diabetic induced experimental animals improved symptoms by reducing the activities of neutrophils to a normal range, therefore stimulating neutrophils to release enzymes like myeloperoxidase and elastase which kill pathogens and modulate inflammation.

Discussion

The present study was carried out to assess therapeutic effect of *Curcuma longa* (turmeric) on some hematological parameters of diabetic induced wistar rats. Hematological assessment is an applied analytical device used in estimating different blood parameters especially those hematological indices considered in this research. The evaluation of these parameters serves as a useful indicator of the health status of animals or humans. Mean Platelet Volume (MPV) is part of complete blood count (CBC). It is a measure of the average platelets size in the blood, and an important biomarker of platelet production, activation as well as provides valuable diagnostic information on the degree of its stimulation in bone marrow and its inflammation, while the standard range values of platelet is maintained at 7.5 –11.5 fL [4]. The induced diabetes mellitus in male rats in this study, is a chronic disease condition due primarily to carbohydrate metabolism disorder which is as a result of ineffectiveness of insulin to regulate blood glucose leading to hyperglycaemia. The condition of diabetes mellitus (hyperglycaemia) in the present study result in platelets becoming less sensitive to inhibitory effect of insulin, leading to increase in activity of mean platelets volume. Abnormal rise in serum concentration of mean platelet volume is often attributed to poor glycemic control [5], accompanied by cardiovascular disease which is linked to platelet hyper-reactivity which is the main index for myocardial infarction [6]. The outcome of this study is in agreement with the reports by [7]; Ayoola [8] and Edem [9] on hyperglycemia, a common cause of platelet hyper-reactivity leading to an increase in mean platelet volume. However, the administration of the extract of *Curcuma longa* in group 1, 2, 3 and metformin (standard anti-diabetic drug) in group 4 marked improvement in the serum level of mean platelet volume in the experimental animals. The administration of the graded doses of *Curcuma longa* ethanolic extracts to the induced groups reduces the activity of serum mean platelet volume in a far better manner as compared to the group treated with standard anti-diabetic drug (metformin).

Serum red cell distribution width together with mean corpuscular volume are valuable hematological indices used in determining the causes of anemia [10]. Damage to red blood cell leads to variations in size or its volume in circulation, and the condition is called Anisocytosis. In this study, the administration of Alloxan followed by the onset of hyperglycemic condition in male wistar rats

causes significant increase in serum red cell distribution width in group 1,2,3,4 and 5 in a dose dependent pattern. The upsurge of serum red cell distribution width is due to severe health conditions like hemolytic anemias, hemorrhage, bone marrow disorders, chronic inflammation, renal/liver disease, cardiovascular disease and defective erythropoietin. Erythropoietin is a glycoprotein with low molecular weight found in the kidney responsible for synthesis, maturation and release of red blood cells from the blood forming tissues, and also ineffective erythropoietin leads to abnormal rise in serum red cell distribution width observed in this study. The administration

of graded doses of rhizomes extract of *Curcuma longa* across the induced groups except group 5, shows a reversal decrease in serum red cell distribution width activities in group 1, 2, 3 with favorable comparison with the group 4 administered with standard anti-diabetic drug (Metformin) and group 6, normal control group respectively. Neutrophils are white blood cells with the defensive role against attacking pathogens like bacteria, fungi and viruses. Neutrophils are characterized by **granulocytes** and cytoplasmic granules surrounded by **myeloperoxidase**, **defensins** (antimicrobial agent) and **lysozyme**. During **phagocytosis**, Neutrophils submerge and digest invading microorganisms (pathogens) and cellular debris with the aid of reactive oxygen species (ROS) and hydrolytic enzymes.

In this research, the hyperglycemic state of groups 1,2, 3, 4 and 5 respectively severely increases the production of neutrophil levels in the experimental animals, and this condition is referred to as neutrophilia. The elevated neutrophil is linked to acute bacterial infections where poor glycemic control reduces the capacity of neutrophil to move to infection spots (chemotaxis), submerge the bacteria or pathogens (phagocytosis) and kill it via reduced degranulation resulting to impair immune response, and therefore exposing diabetic patients to infections. Also, chronic inflammation of the tissues occurs following onset of diabetic condition in the experimental animals, a situation where high glucose level excites chronic oxidative stress, motivating neutrophils to undergo overproduction of reacting oxygen species (ROS) leading to tissue and cardiovascular damages. Thus, this finding is in consonant with Ezeigbo [11], who reported elevated white blood cell counts links to increases in neutrophils concentration. However, the administration of anti-diabetic drug (metformin) result in remarkable decrease in the level of neutrophils in the experimental animals.[12] Similarly, the administration of graded doses of 200mg/kg, 500mg/kg and 700mg/kg of ethanolic *Curcuma longa* extract respectively reduced this parameter to the level comparable to the Un-induce normal control group in a dose- dependent pattern, and this suggested that *Curcuma longa* extract contain anti-diabetic properties such as curcumin, flavonoids and alkaloids which are similar to insulin in lowering or regulating blood glucose levels in the experimental animals therefore improving neutrophil function.[13]

Thickness ratio of the red blood cell is the ratio between the highest thickness of the red blood cell at the periphery and the smallest thickness at the centre leading to the formation of biconcave shape of erythrocytes. The biconcave shape allows red blood cells to increase its surface area/volume ratio for oxygen and carbon dioxide exchange. In the current study, the hyperglycemia condition accelerates elevated thickness ratio of the red blood cell in group I, 2, 3,4 and 5 respectively of the experimental animals.[14] The elevated thickness ratio of the red blood cell in this study is as a result of glycation of red blood cell membrane proteins that distort the normal thickness ratio of red blood cells leading to their inability to maintain the normal biconcave shape. Similarly, abnormal rise in glucose level increases overproduction of reactive oxygen species (ROS) which causes lipid peroxidation of the red blood cell membrane, modification of red blood cell morphology and thickness profile resulting in the increase in thickness ratio of red blood cells. The administration of graded doses of 200mg/kg, 500mg/kg and 700mg/kg of ethanolic *Curcuma longa* extract respectively in the dose dependent manner reduces thickness ratio of the red blood cell to level comparable to the Un-induce normal control group, hence improving red blood cell micro circulation, increased oxygen delivery to tissues as well as reduced risk of cardiovascular complications.[15]

Conclusion

In conclusion, the present study has examined the use of *Curcuma longa* as a plant therapy for the management and treatment of hematological health related challenges like hemolytic anemia, acute bacterial infection, inflammation, myeloproliferation disorder that may arise in patients with acute diabetes. Also, caution should be taken not to consume overdose of *Curcuma longa* since it could drastically reduce the level of red blood cells which may possibly lead to hemolytic anaemia, and reduction in the neutrophils which may cause the patients to be exposed to infections.

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