BLOOD SUGAR REDUCING POTENTIALS AND HEAMATOLOGICAL PARAMETERS OF TURMERIC (*CURCUMA LONGA*) IN ALLOXAN-INDUCED DIABETIC ALBINO RAT (*RATTUS NORVEGICUS*)

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Received *June 15, 2021;* ***Revised*** *June 30, 2021;* ***Accepted*** *July 01, 2021*

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder of glucose metabolism characterized by elevated blood sugar concentrations due to a loss of insulin-producing pancreatic β-cells (type 1 diabetes) or through loss of insulin responsiveness in its target tissues like adipose and muscle (type 2 diabetes). This study was undertaken to investigate the blood sugar reducing potential of turmeric. The duration of the study was 5 weeks and the experimental rats were fed standard rabbit pellet feed and water. Rats were grouped into 6 as follows: Group A - normal control, Group B - diabetic and not treated, Group C - standard control, and Group D – F were diabetic and treated with different doses of turmeric (0, 100, 200 and 400 mg/kg) respectively. Data obtained were analyzed using One-way Analysis Variance (ANOVA). Oral administration of methanolic extract of turmeric led to a significant reduction in blood glucose levels and normalization of other haematological parameters. The extract helped stabilize blood sugar levels and made diabetes more manageable. Standard drug (Glibenclamide) effectively treated the diabetic condition of the experimental rats.

Keywords: Blood sugar, Turmeric, Diabetes, Haematology, Rat

INTRODUCTION

Diabetes is a disease in which the hallmark feature is elevated blood sugar concentrations due to a loss of insulin-producing pancreatic β-cells (type 1 diabetes) or through loss of insulin responsiveness in its target tissues like adipose and muscle (type 2 diabetes) (Schwartz *et al*., 2009). Diabetes affects 5 % of the world population and has become the third human killer after cancer and cardiovascular disease. There is a reservoir of basic information that suggests the involvement of oxidative stress in the pathogenesis of diabetes mellitus. It is now recognized that sustained hyperglycemia in diabetic patients, causes protein glycation and generates free radicals through auto oxidation and polyol pathways (Sharma *et al*., 2000; Ramakrishna and Jailkhani, 2007). High levels of free radicals with concurrent decline of

antioxidant defense mechanisms may lead to the damage of cellular organelles and enzymes (Ottaviano *et al*., 2008). This can culminate in increased lipid peroxidation and development of insulin resistance, which may consequently promote the development of complications of diabetes mellitus (Demozay *et al*., 2008).

Alloxan is used to induce experimental diabetes by selectively destroying pancreatic β-cell. Alloxan is taken up by pancreatic β-cell and subsequently generates reactive oxygen species (ROS),which contributes to DNA fragmentation and evoke other deleterious changes in the cells (Lankin *et al*., 2004) and cell death (Heikkila *et al*., 1976). Abdul-Hamid and Moustafa (2013) reported that alloxan affects directly or indirectly on the membrane potential and ion channels in β-cells.

Alloxan causes necrosis of pancreatic β-cells and induces free radicals which play a relevant role in the etiology and parthenogenesis of both experimental and human diabetes mellitus (Soto *et al*., 2004). It has been suggested that alloxan induces the production of hydrogen peroxide (H_2O_2) and some free radicals such as glutathione disulfide (GSSG) which produce cellular damage followed by cell death. Therefore, alloxan was considered adequate for the study of pathology of diabetes mellitus (Winterbourn and Munday, 1989). Aleeva *et al*. (2002) reported that alloxan decreased the count of insulin producing β-cells, but increased the number of glucagon secreting α-cells in the pancreas, week 1 of diabetes.

Numerous experimental studies have confirmed the important role of naturally occurring phytochemicals in prevention and treatment of diabetes, particularly associated with oxidative stress (Hegazy *et al*., 2013; Leiherer *et al*., 2013; Forcados *et al*., 2017).

Curcuma longa L. commonly known as turmeric has a long history of use in ayurvedic medicine for the treatment of inflammatory conditions (Jurenka, 2009) and a wide variety of diseases including those of the skin, pulmonary and gastrointestinal systems, aches, pains, wounds, sprains and liver disorders (Aggarwal *et al*., 2007). It acts as a scavenger of oxygen free radicals. It can protect haemoglobin from oxidation. In in-vitro studies, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages, which play an important role in inflammation (Shukla and Arora, 2003; Ishita *et al*., 2004). Curcumin also lowers the production of ROS in-vivo (Bagchi, 2012). It has also been used to treat ulcers, parasitic infections, anti-immune diseases and curing symptoms of colds and flus (Siviero *et al*., 2015). Recent studies have shown that curcumin can attenuate cell death caused by oxidative stress, indirectly through induction and/or activation of antioxidant/cytoprotective enzymes, such as haeme oxygenase-1 (HO^{-1}) (Lin *et al*., 2019).

Due to rapid increase in diabetes cases in Nigeria, there is an urgent need for an authoritative, practical algorithm for management of patients with diabetes mellitus.

Curcuminoids based extract are well studied for their pharmacological and safety aspects. Polysaccharide extract of *C. longa* is gaining importance since it showed to have various pharmacological activities, which include antidiabetic, antitumor, antidepressant, antioxidant, antimicrobial/antifertility, hepatoprotective and immunomodulatory properties (Calabrese *et al*., 2003; Mohankumar and McFarlane 2011). This experimental research is geared to verifying the antidiabetic effect of *C. longa* extract in alloxan-induced diabetic albino rat by examining the blood sugar levels as well as changes in the haematological parameters.

MATERIALS AND METHODS

Plant Materials: Fresh rhizomes were procured from Ogbuette Market, Nsukka LGA, Enugu State. The plant and rhizome were identified (Zimdahl, 2018), and authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, where voucher specimen (No: PSB-085) was deposited in their herbarium for reference purposes. The rhizomes were carefully selected and washed off of dirt. The wet weight of the turmeric was weighed (wet weight = 4.5 kg). The rhizomes were cut longitudinally shredded and dried at room temperature until a constant weight was

maintained. The dry shreds were grounded into powder and weighed (dry weight = 0.8 kg).

Turmeric Methanolic Extract: About 0.8 kg of the dry powder of turmeric was placed in a closed vessel and macerated in 800 ml of absolute methanol. The system was allowed to stand for 72 hours with intermittent shaking. The mixture was filtered using Muslin sieve and then with Whatmann filters paper number 42. The filtrate was allowed to dry for 3 days over water bath set at 50^{o}C. Methanol was used as the solvent for extraction because it has a clearer spectrum for extraction and it evaporates easily making the drying process faster.

Experimental Animal: Fourty five (45) adult male albino rats of similar age and weighing 150 ± 2.89 g obtained from Animal Breeding and Genetic Laboratory, Department of Zoology and Environmental Biology, University of Nigeria were used for the experiment. They were given clean water and a standard pellet diet (Specialty Feeds: 19 % crude protein and 14.3 Mj/Kg metabolizable energy) twice daily. Ethical approval for the study (UNN-FBS-2020-081) was obtained from the Faculty of Biological Science Ethical Committee. All investigations were conducted in accordance with the accepted principles for laboratory animal use and care (NRC, 2011).

Acute Toxicity Test: Three groups of healthy albino rats with average weight of 150 ± 2.89 g were used to do toxicity test using OECD method (OECD, 2012). Three animals each were treated with the crude extract of turmeric (300, 900 and 2000 mg/kg) orally. The animals were observed after 24 hour for signs of toxicity and percentage mortality in each group was recorded. The LD_{50} for oral routes was determined by plotting log dose against percentage death but the experiment did not reveal any form of toxicity in the rats. From the experiment carried out, the rats did not die at any of these concentrations

Phytochemical Assay of Turmeric: The assay for phytochemical components of turmeric was adopted from Chanda and Ramachandra (2019).

Alloxan Induction: The weight of the rats using electronic scale and their blood sugar level using a glucometer were measured after an overnight fast. The experimental rats were grouped into 6 with rats in each group. Groups 1 and 2 served as normal and diabetic control respectively, so 5 groups of rats were made diabetic with a single intraperitoneal injection of 120 mg alloxan/kg per body weight. Rats were identified as diabetic on the basis of blood glucose levels ranging above 180 mg/dl after 3 days of induction. The rats were then regrouped according to their diabetic ranges.

Preparation of the Dosage: 0.5 g of the methanolic extract of *C. longa* was weighed and dissolved in 6 ml of 30 % of tween-80 solution, and a required dose of 100, 200 and 400 mg/kg body weight were calculated from the effective dose.

Experimental Design and Administration of Turmeric Extract to Diabetic Rat: The experiment was laid out in a complete randomized design of six treatment groups, replicated thrice with each replicate having two rats. Methanolic extract of turmeric was administered orally to the animals. Group 1 received a standard rat pellet meal and Tween 80 daily, Group 2 served as diabetic control, Group 3 received a dose of 100 mg/kg body weight of turmeric extract, Group 4 received a dose of 200 mg/kg body weight of turmeric extract, Group 5 received a dose of 400 mg/kg body weight of turmeric extract, Group 6 diabetic rats were treated with standard drug (Glibenclamide) at 10 mg/kg body weight. The sub-lethal dosages used were arrived at by dividing the LD_{50} (2000 mg/kg) by a factor (20) to have the initial dose that was subsequently increased by doubling the value.

Collection of Blood: Blood samples were collected from one rat per replicate for haematological studies. The blood samples were taken every week till the end of the experiment. Baseline blood analyses were done before the

administration of the extract commenced. The blood was collected via the orbital sinus using a capillary tube and was put in a clean ethylene diamine tetra acetic (EDTA) bottles to prevent coagulation (Banfi *et al*., 2007). The blood for glucose concentration determination using glucometer method (Togashi *et al*., 2016) was not exposed to EDTA.

Blood Glucose: Blood sample for initial glucose determination just before induction were obtained from the tip of tail of the rats in the groups. The tip of the tail was cut and a little pressure applied to allow for blood flow. A drop of blood was placed on the sensitive part of the glucometer strip (already fixed) and the reading shown on the glucometer was taken. Subsequent blood glucose determination was done with blood samples collected via orbital sinus.

Haematological Parameters

Packed Cell Volume (PCV): PCV volume was determined by the microhaematocrit method. Blood sample was placed in a microcapillary tube, sealed at one end with soap bar and centrifuged at 10000 rpm for 5 minutes using a haematocritcentrifuge. After centrifugation, the PCV were read using a microhaematocrit reader (Sood, 2006).

Haemoglobin Concentration: About 4 ml of haemoglobin reagent (Drapkin's solution) was put in test tube, and 0.02 ml of the blood sample was added and mixed thoroughly. The mixture was allowed to react for 10 minutes and the absorbance were read at 540 nm wavelength against a reagent blank in a spectrophotometer. Standards were prepared as above without blood sample and also read at 540 nm. The haemoglobin concentration of the blood samples was obtained by multiplying the absorbance of the sample with a calibration factor derived from the absorbance and concentration of the standard (Sood, 2006).

Red Blood Cell Count: The red blood cell count was determined using haemocytometer according to the method of Sood (2006). About 0.02 ml of the blood sample was added to 3.95 ml of NaCl in a clean test tube to make a dilution of the blood sample. The diluted blood sample were loaded onto a Neubauer counting chamber and all red blood cells in all the groups of 16 small squares in the central area of the Neubauer chamber were counted using a light microscope at x 40 objectives. The number of cells counted for each sample was multiplied by 10,000 to obtain the red blood cells count per microliter of blood. The derivatives of the RBC were obtained following Baker *et al*. (2001).

White Blood Cell Count: 0.02ml of blood sample was pipetted into a small test tube containing 0.38 ml of white blood cell diluting fluid (Turk's solution) to make 1:20 dilution of the blood sample. The diluted sample were loaded onto the Neubauer counting chamber and all cells on the four-corner square counted using a light microscope at x10 objective, the number of cells counted for each blood sample was multiplied by 50 to obtain the total blood cell count per microliter of blood (Sood, 2006).

Statistical Analysis: Data were analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test to separate the group means. $P<0.05$ was considered significant. The results were reported as means ±standard error of mean (S.E.). All analyses were done using Statistical Package for Social Sciences (SPSS), Version 21, IBM Corporation, Armonk, New York.

RESULTS

Toxicity and Phytochemical Content of Turmeric: No mortality and adverse effects were observed even at the highest dose (2000 mg/Kg) of the turmeric. The phytochemical components of turmeric leaves shows the presence of carbohydrates, proteins, alkaloids, glycosides, terpenes, steroids, flavonoids, tannins, saponins and phenols (Chanda and Ramachandra, 2019).

Effects of Turmeric on the Glucose Levels of Diabetic Rat: The initial glucose levels of the rats recorded significantly highest ($p<0.05$)

values in 400 mg/kg turmeric treated group (97.33 ± 86.06 mg/dL), followed by the diabetic not treated group (83.00 ± 3.75 mg/dL), while the standard control had the lowest mean value of 68.50 ± 2.79 mg/dL (Table 1). The post diabetic induced glucose level of the standard control (474.00 ± 9.59 mg/dL) was the highest, while the normal control had the lowest (83.50 ± 1.20 mg/dL). At Week 1, the glucose level of the diabetic not treated (490.00 ± 42.78 mg/dL) was significantly highest ($p<0.05$), followed by the standard control (437.00 ± 415.68 mg/dL), while the normal control had the lowest (85.33 ± 0.92 mg/dL) glucose levels. The glucose levels of the turmeric treated groups were lower than the standard control and diabetic not treated groups, but higher than the value observed in the normal control group. The diabetic not treated group and the normal control also recorded the highest and the lowest mean value of glucose 335.00 ± 324.65 mg/dL and 86.50 ± 84.78 mg/dL respectively at Week 2. At week 3, the diabetic not treated maintained the highest mean value of glucose (217.00 ± 4.44 mg/dL), followed by the 100 mg/kg turmeric dosed group (203.67 ± 3.39 mg/dL), while the least was recorded in normal control (84.83 ± 1.05 mg/dL), with significant differences ($p<0.05$).

The normal control showed no significant ($p>0.05$) difference in the mean glucose levels across the duration. The standard control and the groups treated with 100 and 400 mg/kg of turmeric had significant ($p<0.05$) duration dependent decrease in glucose from post induction to week 3 respectively. The diabetic not treated group also recorded a significant ($p<0.05$) duration dependent decrease in glucose levels from week 1 to 3 (Table 1).

Effects of Turmeric on the Packed Cell Volume of Diabetic Rat: The mean PCV of the normal (51.33 ± 0.88 %) and standard (46.33 ± 1.20 %) controls recorded significantly highest ($p<0.05$) values when compared with the other groups at Week 1 (Table 2). The PCV result of the induced not treated was the lowest (26.00 ± 1.73 %), while that of the turmeric treated groups did not differ significantly ($p>0.05$) from each other. At week 2, the normal control recorded the highest PCV of 50.00 ± 1.15 %, while the standard control had the least (24.50 ± 0.29 %). The turmeric treated groups had significant ($p<0.05$) dose-dependent decrease in PCV values at Week 2. At Week 3, the normal control and induced not treated group recorded the highest (51.33 ± 1.33 %) and the lowest (32.33 ± 1.20 %) mean PCV values respectively with significant differences ($p<0.05$).

The PCV values of the normal control was not significantly different ($p>0.05$) across the week. The weekly comparison of the PCV results showed a significant decrease and increase ($p<0.05$) in PCV at Weeks 2 and 3 respectively in standard control, 200 and 400 mg/kg turmeric treated groups. The induced not treated and 100 mg/kg turmeric treated group showed a significant increase and decrease ($p<0.05$) in PCV at Weeks 2 and 3 respectively (Table 2).

Effects of Turmeric on the Haemoglobin Levels of Diabetic Rat: The normal and standard controls recorded significantly higher ($p<0.05$) mean Hb values compared with the other treatment groups at week 1 (Table 3). The diabetic untreated group had the lowest mean Hb value (6.54 ± 0.169 g/dL), followed by the group treated with 100 mg/kg of turmeric (8.46 ± 0.64 g/dL) and 200 mg/kg (8.46 ± 0.22 g/dL) that did not differ significantly ($p>0.05$) from each other. At Week 2, the mean of Hb in normal control did not differ significantly ($p>0.05$) from the groups treated with 100, 200 and 400 mg/kg of turmeric. The normal control had the highest mean Hb (10.72 ± 0.29 g/dL), followed by the standard control (9.73 ± 0.28 g/dL), while the diabetic not treated had the lowest Hb value (4.71 ± 0.39g/dL). The mean Hb concentrations of the turmeric treated groups were dose-dependent at Week 3.

Comparing the Hb values across the weeks revealed no significant difference ($p>0.05$) in the mean Hb value of rat in the normal control.

Table 1: Effects of methanolic extract of turmeric rhizome on the glucose levels (mg/dl) of diabetic albino rat

Treatment groups	Concentration (mg/kg)	Glucose level (mg/dl)				
		Initial	Post induction	Week 1	Week 2	Week 3
Normal control	-	82.83 ± 1.51^{b1}	83.50 ± 1.20^{a1}	85.33 ± 0.92^{a1}	86.50 ± 84.78^{a1}	84.83 ± 1.05^{a1}
Standard control (Glibenclamide)	10	68.50 ± 2.79^{a1}	474.00 ± 9.59^{e4}	437.00 ± 415.68^{d4}	263.00 ± 228.51^{c3}	147.00 ± 5.79^{b2}
Diabetic untreated (Tween 80)	0	83.00 ± 3.75^{b1}	408.50 ± 7.48^{d34}	490.00 ± 42.78^{d4}	335.00 ± 324.65^{e3}	217.00 ± 4.44^{d2}
Diabetic treated with turmeric	100	76.50 ± 67.57^{ab1}	369.00 ± 12.90^{cd4}	324.67 ± 3.31^{c4}	291.50 ± 10.96^{d3}	203.67 ± 3.39^{d2}
Diabetic treated with turmeric	200	73.33 ± 63.19^{ab1}	252.83 ± 13.83^{b4}	206.67 ± 3.10^{b3}	232.00 ± 5.37^{b4}	178.00 ± 1.46^{c2}
Diabetic treated with turmeric	400	97.33 ± 86.06^{c1}	335.33 ± 40.64^{c5}	299.67 ± 28.81^{c4}	242.50 ± 14.98^{bc3}	181.33 ± 10.05^{c2}

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05); Mean values with different numbers as superscripts in a row differ significantly (p<0.05)

Table 2: Effects of methanolic extract of turmeric rhizome on the packed cell volume levels of diabetic albino rat

Treatment groups	Concentration (mg/kg)	Packed cell volume (%)		
		Week 1	Week 2	Week 3
Normal control	-	51.33 ± 0.88^{c1}	50.00 ± 1.15^{e1}	51.33 ± 1.33^{c1}
Standard control (Glibenclamide)	10	46.33 ± 1.20^{bc2}	24.50 ± 0.29^{a1}	46.67 ± 1.45^{b2}
Diabetic not treated (Tween 80)	0	26.00 ± 1.73^{a1}	43.50 ± 0.29^{c3}	32.33 ± 1.20^{a2}
Diabetic treated with turmeric	100	41.00 ± 1.15^{b1}	47.00 ± 0.58^{d3}	44.33 ± 0.88^{b2}
Diabetic treated with turmeric	200	41.00 ± 1.00^{b1}	41.50 ± 0.87^{bc1}	45.67 ± 1.20^{b2}
Diabetic treated with turmeric	400	44.00 ± 3.06^{b2}	41.00 ± 0.58^{b1}	45.67 ± 0.33^{b2}

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05); Mean values with different numbers as superscripts in a row differ significantly (p<0.05)

Table 3: Effects of methanolic extract of turmeric rhizome on the haemoglobin levels of diabetic albino rat

Treatment groups	Concentration (mg/kg)	Haemoglobin level (g/dl)		
		Week 1	Week 2	Week 3
Normal control	-	11.40 ±0.20^{d2}	9.71 ±0.29^{d1}	10.72 ±0.29^{b12}
Standard control (Glibenclamide)	10	10.83 ±0.30^{d2}	7.05 ±0.45^{a1}	9.73 ±0.28^{cb2}
Diabetic not treated (Tween 80)	0	6.54 ±0.17^{a2}	7.42 ±0.07^{bc2}	4.71 ±0.39^{a1}
Diabetic treated with turmeric	100	8.46 ±0.64^{b1}	8.50 ±1.53^{c1}	7.32 ±0.43^{b1}
Diabetic treated with turmeric	200	8.46 ±0.64^{b1}	9.05 ±0.22b^{c1}	8.87 ±0.50^{b1}
Diabetic treated with turmeric	400	9.63 ±0.35^{bc1}	9.09 ±0.26^{ab1}	9.25 ±0.68^{c1}

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05); Mean values with different numbers as superscripts in a row differ significantly (p<0.05)

The Hb value of rat in the standard control decreased at week 2(7.05 ± 0.45 g/dL), and increased at week 3(9.73 ± 0.28 g/dL), with significant differences ($p<0.05$) respectively.

The Hb values of weeks 1 and 2 of the diabetic not treated group did not differ significantly ($p>0.05$) from each other, but varied significantly with the value recorded at week 3 (4.71 ± 0.39 g/dL). The Hb concentrations of the turmeric treated groups varies across the weeks, but did not differ significantly ($p>0.05$) (Table 3).

Effects of Turmeric on the Red Blood Cell Counts of Diabetic Rat: The normal and standard controls recorded significantly higher ($p<0.05$) mean RBC values, while the diabetic not treated group recorded the lowest (3.81 ± 0.29 x 10^{12}/L) RBC counts when compared with other treatment groups at week 1 (Figure 1).

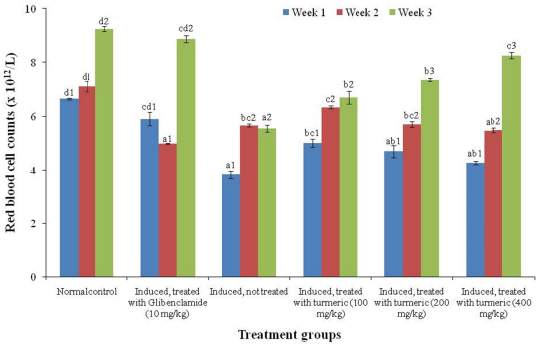

Figure 1: Effects of turmeric on the red blood cell counts of diabetic rat. *Values with different alphabetic superscript are significantly different ($p<0.05$) between treatment groups within the same duration. Values with significant numeric superscript differ significantly ($p<0.05$) between duration within the same treatment group*

The various turmeric treated groups had significant ($p<0.05$) dose-dependent decrease in RBC values at week 1. The normal control had the highest significant ($p<0.05$) mean value of RBC (7.11 ± 0.39 x 10^{12}/L), while the standard control recorded the lowest (4.99 ± 0.34 x 10^{12}/L) at week 2. There was a gradual significant ($p<0.05$), dose-dependent decrease in RBC counts among the turmeric treated groups at week 2. The RBC counts recorded at week 3 was highest in normal control (9.25 ± 0.17x 10^{12}/L) and standard control (8.87 ± 0.27 x 10^{12}/L), and lowest in diabetic untreated group (5.56 ± 0.26 x 10^{12}/L), with significant differences ($p<0.05$). The mean RBC concentrations of the turmeric treated groups were dose-dependent at week 3.

The normal control rats had gradual increase in RBC counts across the weeks with a significant difference ($p<0.05$) at week 3, the standard control decreased at week 2, and increased significantly ($p<0.05$) at week 3. The diabetic untreated group increased significantly ($p<0.05$) at week 2, and did not vary significantly ($p>0.05$) at week 3. All the turmeric feed groups gradually increased significantly ($p<0.05$) across the weeks (Figure 1).

Effects of Turmeric on the White Blood Cells Count of Diabetic Rat: The diabetic not treated group had the highest significant ($p<0.05$) mean value of WBC (9600.00 ± 416.33 x 10^9/L), while the normal control recorded the lowest (6433.33 ± 317.98 x 10^9/L) at week 1. The WBC results of the turmeric feed groups did not vary significantly ($p>0.05$). At week 2, the standard control had the highest mean WBC value of 12000.00 ± 346.41 x 10^9/L, while the normal control recorded the lowest (6700.00 ± 57.73 x 10^9/L), with significant ($p<0.05$) differences. There was a significant ($p<0.05$), dose-dependent increase in RBC results among the turmeric feed groups at Week 2. The diabetic not treated group recorded the highest significant ($p<0.05$) mean value of WBC (11000.00 ± 416.33 x 10^9/L), while the normal control recorded the lowest (5866.67 ± 176.38 x 10^9/L) at week 3 (Table 4).

The normal control did not show any significant ($p>0.05$) difference in WBC across the Weeks, while the standard control increased at Week 2 and decreased at week 3 (6300.00 ± 208.17 x 10^9/L) with significant differences ($p<0.05$). The WBC value of the diabetic not treated increased significantly ($p<0.05$) at Week 3. The group feed 100 mg/kg of turmeric recorded a reduction in mean WBC at Week 2 (7350.00 ± 144.34 x 10^9/L) and an increase at week 3 (8466.67 ± 545.69 x 10^9/L), while the 200 and 400 mg/kg turmeric feed groups recorded an increase at week 2 (8600.00 ± 115.47 x 10^9/L) and (9600.00 ± 115.47 x 10^9/L), and a decrease at Week 3 (7300.00 ± 264.58 x 10^9/L) and (7733.33 ± 176.38 x 10^9/L), with significant ($p<0.05$) differences respectively (Table 4).

Table 4: Effects of methanolic extract of turmeric rhizome on the white blood cell levels of diabetic albino rats

Treatment groups	Concentration (mg/kg)	White blood cell counts (x 109/L)		
		Week 1	Week 2	Week 3
Normal control	-	6433.33 ± 317.98^{a2}	6700.00 ± 57.73^{a3}	5866.67 ± 176.38^{a1}
Standard control (Glibenclamide)	10	7100.00 ± 173.20^{ab1}	120000.00 ± 346.41^{e2}	6300.00 ± 208.17^{ab1}
Diabetic not treated (Tween 80)	0	9600.00 ± 416.33^{d1}	9500.00 ± 173.21^{d1}	11000.00 ± 416.33^{e2}
Diabetic treated with turmeric	100	8866.67 ± 405.51^{cd2}	7350.00 ± 144.34^{b1}	8466.67 ± 545.69^{d2}
Diabetic treated with turmeric	200	8166.67 ± 185.59^{bc2}	8600.00 ± 115.47^{c2}	7300.00 ± 264.58^{bc1}
Diabetic treated with turmeric	400	7966.67 ± 425.57^{bc1}	9600.00 ± 115.47^{d2}	7733.33 ±176.38^{cb1}

Mean values with different alphabets as superscripts in a column differ significantly ($p<0.05$); Mean values with different numbers as superscripts in a row differ significantly ($p<0.05$)

DISCUSSION

Turmeric is a well-known condiment, which is used in our daily diet and has many medicinal properties (Santoshkumar *et al.*, 2013).The research aimed at evaluating the anti-diabetic effect of turmeric which is rich in active ingredient - curcumin.

Investigations of acute toxicity study revealed no signs and symptoms or toxicity or mortality in any of the animals even at a very high dose. Consequently, the present study indicated that turmeric has no toxic effects in rats. This result was in agreement with the study of Aggarwal *et al*. (2016) on of the non-toxicity of curcuminoid-essential oil complex (a bioavailable turmeric formulation) in rats. Therefore, following investigations of acute toxicity, turmeric was safe, non-toxic pharmacological formulation.

Turmeric contains a wide variety of phytochemicals, including curcumin, turmerin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, eugenol, triethylcurcumin, zingiberene, curcumenol, curcumol, turmerones and turmeronols. Among these phytochemicals, the most active component of turmeric is curcumin which gives a yellow color to turmeric and responsible for most of the therapeutic effects (Adinew, 2012). Other phytochemical components of turmeric are carbohydrates, proteins, alkaloids, tannins, saponins, glycosides, terpenes, steroids, flavonoids and phenols (Chanda and Ramachandra, 2019).

Research carried out by Santoshkumar *et al.* (2013) showed that turmeric extract 300 mg/kg significantly reduced the blood glucose levels in diabetic rats from 7th day onwards. But 500 mg/kg had more efficient action, as reduction in blood glucose levels was statistically highly significant. This experiment showed significant dose-dependent decrease in blood sugar in turmeric treated groups. Glibenclamide, a standard anti-diabetic drug, reduced blood glucose by a highly significant level. The possible mechanism of the effect of curcumin on glycaemia in diabetes models may be because curcumin could attenuate tumor necrosis factor-α (TNF-α) levels (El-Azab *et al*., 2011).

There are various mechanisms for anti-diabetic effect of curcumin. One of the most fundamental of these mechanisms is that improvement in beta-cells function through its anti-inflammatory and anti-oxidant properties (Rivera-Mancía *et al*., 2015). In addition curcumin reduces fasting blood glucose by increasing peroxisome proliferator-activated receptor (PPAR) activity, inhibits hyperglycemic, increases glycolysis, inhibits liver gluconeogenesis, stimulations secretion insulin from pancreas, provokes glucose uptake by increasing gene expression of GLUT4, GLUT2 and GLUT3, suppresses liver production through improvement AMP kinase activation and inhibits glucose 6 phosphate kinase (Hodaei *et al*., 2019). On the other hand curcumin increases the expression of adiponectin genes and thus leads to raising insulin sensitivity.

In this study, oral administration of turmeric extract caused significant changes in PCV of rats comparatively throughout the weeks of treatment. PCV is the measurement of the proportion of blood that is made up of cells. The value is expressed as a percentage or fraction of cells in the blood. For example, a PCV of 40 % means that there are 40 milliliters of cells in 100 milliliters of blood (Fairbanks and Tefferi, 2000). This experiment showed that PCV decreased significantly in diabetic and untreated rat as well as 100 mg/kg turmeric treated rat, while it increased with higher concentration of turmeric extract (200 and 400 mg/kg) and rats treated with standard drug (Glibenclamide). A research carried out by Jaman *et al*. (2018) showed that PCV decreased significantly in diabetic patients (HbA1c ≥ 75) when compared with non-diabetic patients (HbA1c ≤ 6.5). The decreased value of diabetic patient may be due to the dehydration and accumulation of protein.

Haemoglobin is known as a protein in red blood cells that helps store and transport oxygen. The turmeric treated rat groups had considerable low Hb when compared to the normal and standard group which showed alterations across the research period. The diabetic not treated had the lowest Hb value throughout the research period. This supports the fact that diabetic nephropathy and diabetic retinopathy result in increased susceptibility to low haemoglobin levels (Ranil *et al*., 2010).

Alloxan exposure resulted in slight decreases in RBC and HB levels all of which may lead to anaemia. Reduction in the RBC counts may be attributed to hyperactivity of the bone marrow, leading to the production of red blood cells with impaired integrity which were easily destructed in the circulation or also termed as atrophied erythrocytes (Hossen *et al*., 2017). Our findings suggest that the decrease in RBC counts (i.e. microcytic hypochromic anaemia) occur due to excessive damage to the erythrocytes or inhibition of erythrocyte formation as also reported by Elsharkawy *et al*. (2013). However, administration of turmeric increased the RBC and HB levels, indicating immune stimulatory activity of the extract (Hossen *et al*., 2017) rendering turmeric as a potential alternative armamentarium against anaemia.

This research also showed decrease in RBC of the diabetic not treated rats and in turmeric treated rat relative to rats in the normal and standard group. There was significant increase in all groups at week 3, this could be due to continual production of RBC in the bone marrow. The increase in the RBC count of rats with increased concentration of turmeric may be associated with the effects of turmeric bioactive compounds on improving antioxidant status of the rats (Amalraj *et al*., 2017). Tuntipopipat *et al.* (2009) noted a case of a possible human iron deficiency due to the absorption of iron in the gut due turmeric. While causality cannot be readily determined, the patient's haemoglobin, iron and ferratin were reduced after turmeric was started and went back to normal after it was stopped. Administration of turmeric to the 3 treated groups possibly contributed to low Hb levels.

White blood cell count was highest in infected untreated group, less in turmeric treated groups and least in the standard and normal group. According Srinivasan *et al.* (2003), glucose causes significant production of interleukin 8, a potent chemo-attractant that may be responsible for recruitment of neutrophils by human endothelia cells. That is to say that hyperglycemia causes high level of white blood cells. High white blood cell count may indicate that the immune system is working to destroy the infection, it may also be a sign of physical or emotional stress as in the case of diabetes oxidative stress.

Conclusion: Turmeric is a promising medicinal plant which can be used as an adjunct to drug and diet therapy for the management of diabetes mellitus. Curcumin is a potent antioxidant that can neutralize free radicals due to its chemical composition and structure. Curcumin supplements should be taken at early stages of diabetes to prevent further oxidative damage and production of reactive oxygen species associated with diabetes. Daily dose of 200 to 400 mg of turmeric is recommended in preparing diet for diabetic patients to help in lower and stabilize their blood sugar.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Felix Nwankwo of the Plant Science and Biotechnology, University of Nigeria, Nsukka, for assisting in identification and authentication of the plant rhizome. We also thank the technical and laboratory staff of Animal Breeding and Genetic Laboratory, for maintenance of the animals.

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