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Aspirin and meloxicam co-administration with leaf extract of *Terminalia catappa*; prevention of red blood cell related cardiovascular complications in alloxan induced diabetic rats

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Abstract

Hyperglycemia induced alterations in red blood cell parameters are associated with cardiovascular complications in diabetes mellitus. This study was designed to investigate abnormalities in red blood cell parameters and changes due to aspirin and meloxicam administrations in diabetic rats. Thirty-six (36) healthy male Wistar rats weighting 150-200g were used for the study. The animals were randomly distributed into six groups of six rats each. Group 1 and 2 respectively served as control and diabetic groups administered with 5 ml/kg body weight of distilled water orally. Groups 3 and 4 were diabetic rats treated with aspirin and meloxicam at 30 mg/kg and 2 mg/kg body weight respectively. A combined administration of aspirin and meloxicam at their respective doses with 130mg/kg body weight of *Terminalia catappa* extract was in groups 5 and 6. The results showed that RBC count, PCV, HGB and MCV were reduced significantly ($p<0.05$) in diabetic group but significantly ($p<0.05$) increased in aspirin, meloxicam and in combined extract treated groups of diabetic rats. Therefore, diabetic induced alterations in red blood cell parameters which maybe attributed to inflammation were reversed by aspirin and meloxicam. Co-administration of these NSAID with *T. catappa* leaf extract would enhance their safety use in prevention of cardiovascular complications in diabetes mellitus.

Keywords: Red blood cell; diabetic anaemia; *Terminalia catappa*; Aspirin; Meloxicam; Inflammation; Cardiovascular complications

1. Introduction

Haematological abnormalities are common among people with diabetes mellitus. These abnormalities affect all cell type which include red blood cell, white blood cell and platelets [1]. Due to the possible major impact of these haematological issues on diabetic patients, it becomes imperative to diagnose such and administer appropriate timely treatment. It is well established that anaemia and other red blood cell related abnormalities exist in diabetes mellitus [1]. Anaemia is one of the commonest and prevalent blood-related disorder which occurs in patients with diabetes mellitus (DM) [2]. But these complications of diabetes mellitus are often neglected and untreated [3,4]. Growing evidence indicates that anaemia in T2DM patients is a strong and independent indicator of increased risk for diabetes-related macrovascular and microvascular complications [5,6,7]. Also sustained hyperglycaemia, as seen in type 1 diabetes, has been shown to induce cardiovascular complications through haematological alterations which include reduced deformability of the erythrocyte and increased haemolysis [8,9,10]. Findings from many studies in diabetic clinics have brought to bear the prevalence of unrecognized anaemia as being nearly two to three folds greater than the general population [11,12,13,14]. Moreover, DM patients tend to develop anaemia at earlier ages with greater severity than the general population, and they are therefore exposed to greater risk of complications [15,16,17,18,19,20]. Anaemia contributes

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to early occurrence and rapid progression of complications like diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, end-stage renal diseases, ischemic heart disease, and non-healing diabetic foot ulcers [21].

The presence of anaemia in DM patients could be due to shortened life span of red blood cell [22] or renal impairment [23,24] influencing the availability of erythropoietin [25] which is a major hormone involved in erythropoiesis. However, there are evidence of the risk of early occurrence of anaemia in diabetic patients without renal impairment suggesting the availability of other aetiologies of anaemia in diabetes mellitus [26,27,24]. Besides the disturbances in the availability or action of this hormone, other causes may result in the elevation of internal viscosity and increased membrane rigidity in these blood cells [28] predisposing them to haemolysis.

Involvement of inflammation in the pathophysiology of diabetes mellitus is well-established [29] and aspirin has been used as non-steroidal anti-inflammatory drug (NSAID) for treatment in diabetic complications including cardiovascular complications [30,31]. However, the use of aspirin of recent is generating a lot of controversy amidst claimed benefits. According to 2019 guidelines, low dose (75 – 100 mg/day) aspirin was recommended for primary prevention in people with type 2 diabetes while others advocated for use in preventive therapy in secondary cardiovascular condition [32]. Recent meta-analysis report showed that both low and high dose aspirin therapy is associated with overwhelming disadvantages such as ischemic stroke, haemorrhagic stroke, upper gastrointestinal bleeding and intracerebral bleeding [33,34,35,36]. Therefore, co-administration of non-steroidal anti-inflammatory drugs with *T. catappa* extract was investigated on red blood cell parameters in diabetes mellitus.

2. Material and Methods

2.1. Preparation of Plant extract

Fresh leaves of *Terminalia catappa* were collected at the premises of the University of Uyo and authenticated by a botanist at the Department of Botany and Ecological studies, University of Uyo with reference number UUPH 22(a). The leaves were washed with clean water to remove debris. The water was blotted out and kept overnight at room temperature to dry up. The clean leaves were pulverized and 5000 g of the pulverized leaves was soaked in 5 litres of deionised water for 18 hours. The mixture was filtered using muslin cloth and evaporated to dryness using thermostatic water bath at 45 ℃ until a semi solid paste was obtained. 204.18 g of the extract was obtained after evaporation which represent a percentage yield of 4.08 % and the extract was stored in refrigerator for later use.

2.2. Preparation of Experimental animal

Thirty-six (36) healthy male Wistar rats weighting between 150-200 g were used for the study. The animals were procured from the animal house, Faculty of Basic Medical Sciences, University of Uyo and were housed in a well-ventilated cage in the animal house. They were allowed to acclimatize for two weeks and maintained in a 24-hour dark and light cycle. The animals were fed with standard pellets (from Guinea Feeds, Plc Nigeria) and have access to water ad libitum.

2.3. Induction of Diabetes

Diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/Kg body weight [37,38,39] The animals were assessed for development of diabetes after 72 hours [40] by obtaining blood sample from the tip of the tail. The blood sample was dropped into glucose strip to measure the glucose level using a glucometer (One Touch Ultra, Life Scan Inc, U.S.A). Blood glucose of ≥200 mg/dl was considered diabetic (normal range of blood glucose in rat is 80–120 mg/dl) and were used for the experiments [38,40].

2.4. Experimental design

The experimental animals were randomly distributed into six (6) groups of six (n=6) rats per group and the experiment was conducted as follows:

- Group 1: Control group administered with only distilled water orally at a dose of 5 ml/kg body weight.
- Group 2: Diabetic group administered with only distilled water orally at a dose of 5 ml/Kg body weight.
- Group 3: Diabetic group treated orally with 30 mg/kg body weight of aspirin.
- Group 4: Diabetic group treated orally with 2 mg/kg body weight of meloxicam.
- Group 5: Diabetic group treated orally with 30 mg/Kg of aspirin and 130 mg/Kg body weight of *Terminalia catappa*

- Group 6: Diabetic group treated orally with 2 mg/Kg of meloxicam and 130 mg/Kg body weight of *Terminalia catappa*

2.5. Collection of blood samples

At the end of the experiment, blood samples were obtained from the experimental animal 24 hours after the last administration. The animals were sedated with chloroform vapour and blood was collected by cardiac puncture. Blood sample from each animal was emptied into EDTA sample sequestered bottle. The bottles were gently turned to mix adequately with the anticoagulant.

2.6. Determination of haematological parameters

Various red blood cell parameters were determined using auto-haematological analysis method. Blood from each sequestered bottle used and standard laboratory procedures for auto-haematological analysis were employed using Midray haematological auto analyser (model BC5300, serial number OA-101505, Germany). The result for each parameter was obtained in a print out from the analyser.

2.7. Statistical analysis

Data were analysed using Microsoft Excel and Graphpad Prism statistical software version 5.0. Results were represented using tables and graphs. Descriptive analysis i.e. comparison of mean was by analysis of variance (ANOVA) with a post hoc test. The results were presented as mean ± standard error of mean (SEM) and the values at $p<0.05$ were considered significant

3. Result

3.1. Red Blood Cell Count

The results of red blood cell count are represented in figure 1. The results showed that the red blood cell count was 6.65±0.49 x 10^12/cm in the control group. The value was reduced significantly ($p<0.05$) in diabetic group to 5.31±0.11 x 10^12/cm when compared with control group. The diabetes+aspirin and diabetes+meloxicam group were 6.72±0.31 x 10^12/cm and 6.70±0.29 x 10^12/cm respectively. These values were not significantly different from control group but comparing it with diabetic group showed significant difference. In the diabetic group administered with a combined extract with aspirin and meloxicam has mean value of 7.51±0.12 x 10^12/cm and 7.04±0.21 x 10^12/cm respectively. The values were not significantly different from the control but was significantly ($p<0.05$) diabetic group. It is observed that they are also significantly different from the aspirin treated group.

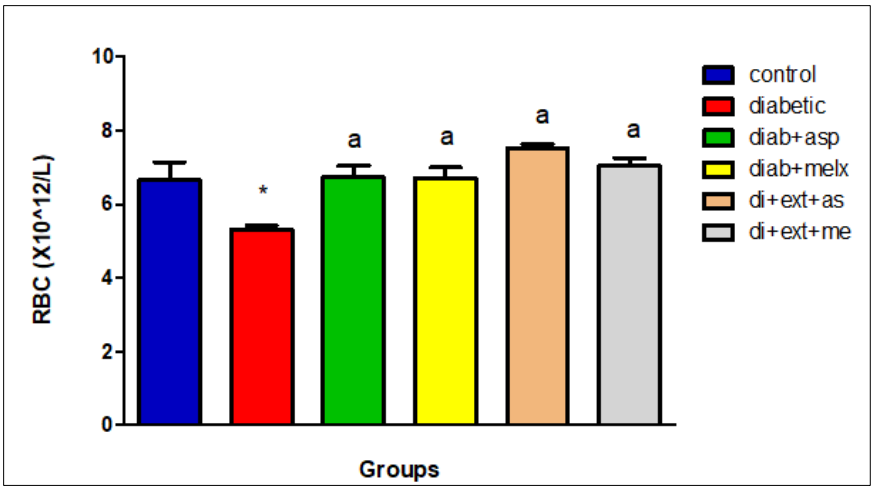

Figure 1 Red blood cell count in control and diabetic treated groups. Values are in mean ± SEM, $p<0.05$. *=test vs control group, a=test vs diabetic group

3.2. Haemoglobin (HGB) Level

The results of haemoglobin level are as shown in figure 2. The haemoglobin level in the control group was 10.96±0.35 mg/dl while the diabetic group was 9.05±0.17 mg/dl which showed significant ($p<0.05$) reduction compared with

control group. The aspirin and meloxicam treated groups with haemoglobin levels of 11.34±0.33 mg/dl and 10.98± respectively were not changed significantly when compared with the control. Similarly, PCV of diabetic groups with combined treatment of extract+aspirin and extract+meloxicam of 12.2±0.15 mg/dl and 12.27±0.14 mg/dl were significantly higher than diabetic group but no significant difference with control group

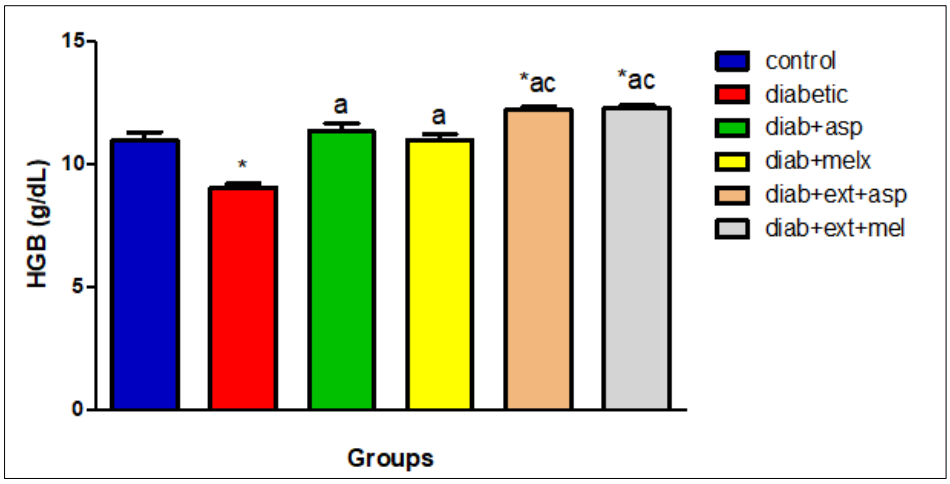

Figure 2 Heamoglobin concentration in control and diabetic treated groups. Values are in mean ± SEM, p<0.05. *=test vs control group, a= test vs diabetic group, c= test vs diabetic + meloxicam group

3.3. Haematocrit (HCT)

The results of HCT is shown in figure 3. The diabetic group had HCT of 29.85±0.65 %, a value significantly (p<0.05) lower than 39.8±0.99 % in the control group. The HCT values for diabetes+ aspirin, diabetes+ meloxicam, diabetes+ extract+ aspirin and diabetes+ extract+ meloxicam was 42.7±1.35 %, 39.94±0.73 %, 43.03±0.55 % and 42.2±0.54 % respectively. These values were all significantly (p<0.05) increased compared to diabetic group but marginally higher than the control group value.

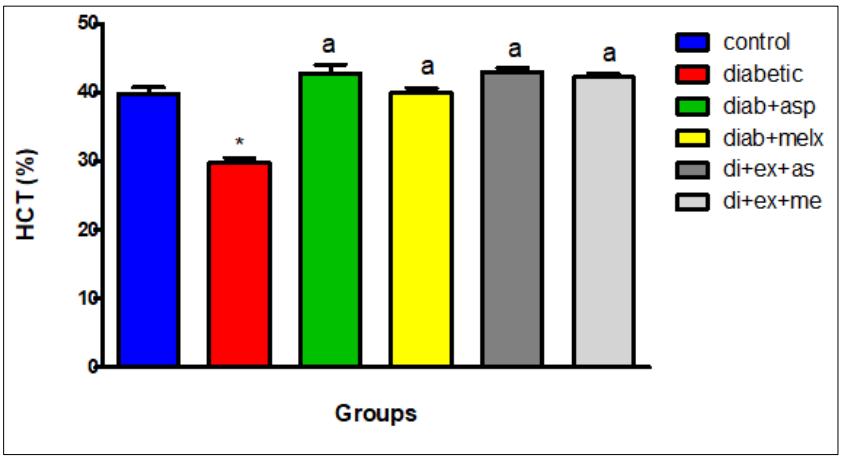

Figure 3 Heamatocrit in control and diabetic treated groups. Values are in mean ± SEM, p<0.05. *=test vs control group, a= test vs diabetic group

3.4. Mean Corpuscular Volume (MCV)

In figure 4, the result showed mean corpuscular volume in diabetic group to be 56.37±0.71 fL, significantly (p<0.05) reduced compared to MCV of 61.64±1.15 fL in the control group. Diabetic group treated with aspirin presented an elevated MCV of 63.46±1.65 fL which increases marginally above control group but was significantly (p<0.05) increased above the diabetic group. The MCV of diabetic groups treated with meloxicam, extract+aspirin and extract+meloxicam was 59.5±0.98 fL, 57.33±0.33 fL, and 59.87±1.23 fL respectively. These values were not significantly different from both control and diabetic groups. However, it was observed that the MCV in extract+aspirin group was significantly (p<0.05) lower than diabete+aspirin treated group.

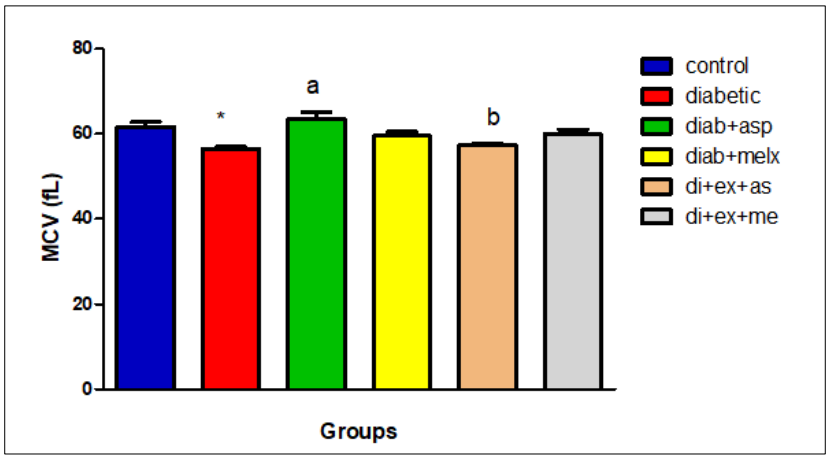

Figure 4 Mean corpuscular volume in control and diabetic treated groups. Values are in mean ± SEM, p<0.05. *= test vs control group, a= test vs diabetic group, b= test vs diabetic + aspirin group

3.5. Mean Corpuscular Haemoglobin (MCH)

The mean corpuscular haemoglobin concentration (figure 5) shows that the control group value was 17.08±0.51 Pg while the diabetic group was 17.22±0.14 Pg and they were not significantly different from each other. The diabetic group treated with aspirin and meloxicam as well as the combinations diabetes+extract+aspirin and diabetes+extract+meloxicam was 16.84±0.22 Pg, 16.42±0.25 Pg, 16.27±0.08 Pg and 17.40±0.35 Pg respectively. These values were not significantly different from the control and among the group.

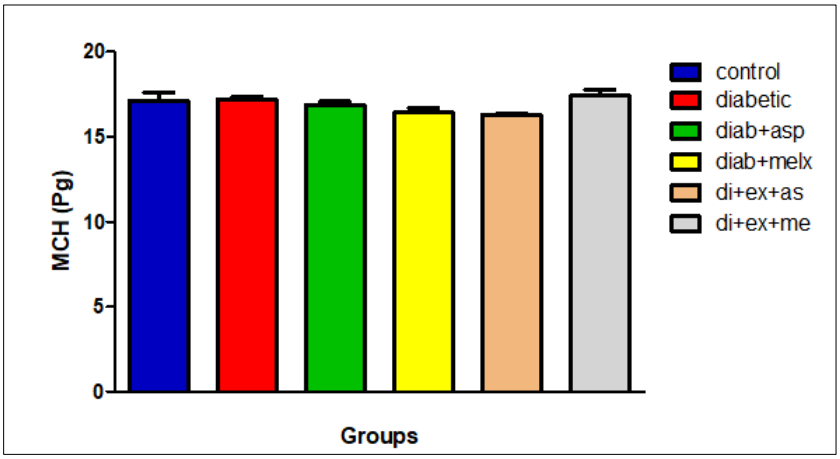

Figure 5 Mean corpuscular haemoglobin in control and diabetic treated groups. Values are in mean ± SEM, p<0.05

3.6. Mean Corpuscular Haemoglobin Concentration (MCHC)

Figure 6 represents the results of MCHC which were 27.72±0.60 g/dl for control group, 30.60±0.29 g/dl for diabetic group, 26.62±0.50 g/dl for aspirin group and 27.72±0.76 g/dl for meloxicam group. The others were diabetic+extract+aspirin and diabetic+extract+ meloxicam with values 28.33±0.06 g/dl and 29.07±0.32 g/dl respectively. The diabetic group was significantly (p<0.05) increased in comparism with control group while the aspirin, meloxicam and extract+aspirin groups were not significantly different from control but was reduced significantly (p<0.05) when compared with diabetic group. Comparing other groups with the aspirin treated group only diabetic+extract+ meloxicam group showed significant (p<0.05) difference.

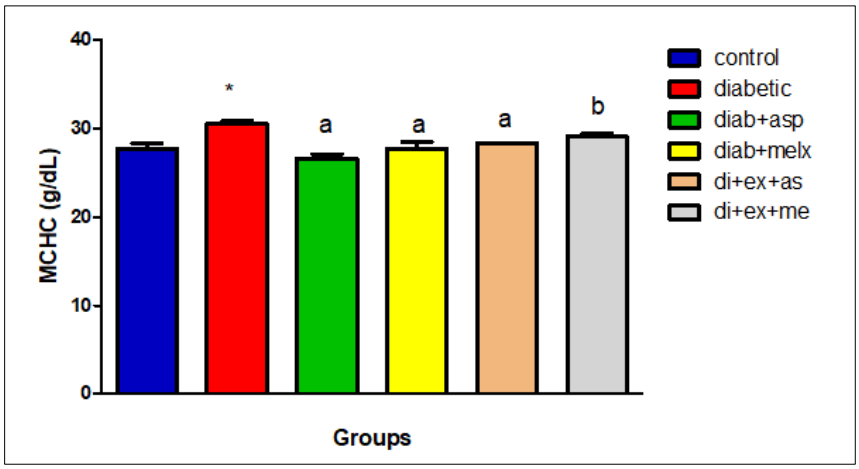

Figure 6 Mean corpuscular haemoglobin conc. in control and diabetic treated groups. Values are in mean ± SEM, p<0.05. *= test vs control group, a= test vs diabetic group, b= test vs diabetic + aspirin group.

3.7. Red Cell Distribution Width-CV

The results of red cell distribution width (RDW-CV) as shown in figure 7 had control group value of 0.17±0.01 %. diabetic group was 0.17±0.01 %, diabetic+aspirin was 0.17±0.01 % while diabetic+meloxicam was 0.19±0.1 %. the diabetic groups administered with a combination of extract+aspirin and extract+meloxicam has 0.19±0.01 % and 0.17±0.01 % respectively. No significant changes were observed in all the test groups compared with either control or diabetic group.

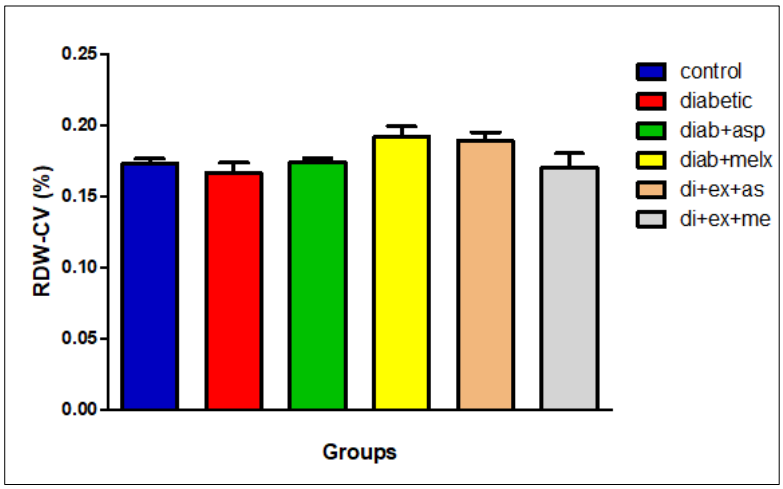

Figure 7 Red cell distribution width-CV in control and diabetic treated groups. Values are in mean ± SEM, p<0.05

3.8. Red cell distribution width–SD

The results in figure 8 represents the red cell distribution width (RDW-SD). In control group the value was 43.53±0.77 fL and reduced slightly to 38.82±1.37 fL. This reduction was however not significant. The aspirin treated group value was 45.10±1.12 fL and meloxicam treated group was 46.28±1.67 fL and these were significantly increased compared to the diabetic group but no difference was statistically observed with the control group. The values of combined administration of extract+aspirin and extract+meloxicam were 44.2±1.91 fL and 41.77±0.94 fL which were not significantly different from the control and diabetic groups.

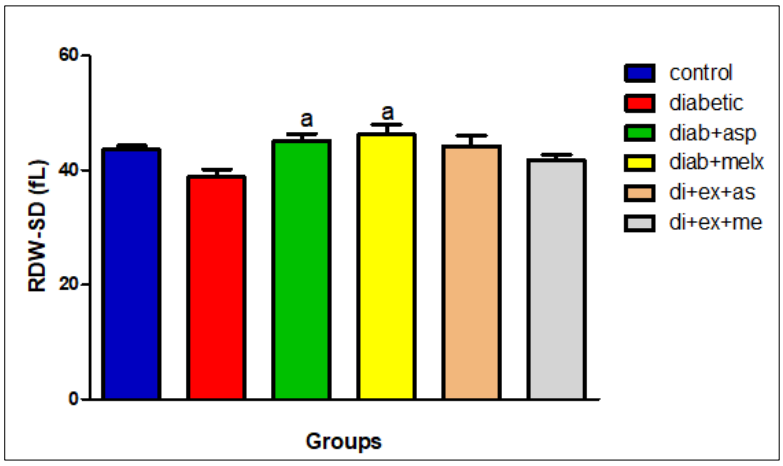

Figure 8 Red cell distribution width-SD in control and diabetic treated groups. Values are in mean ± SEM, p<0.05. *= test vs control group, a= test vs control group, a= test vs diabetic group

4. Discussion

The research report on haematological parameters associated with diabetes mellitus in this study focused on red blood cells and its associated parameters. Red blood cells play an essential role in the delivery of oxygen to body tissues throughout the circulatory system [41]. Changes in the red blood cell count is predictive of certain disease condition as well as influence of some drugs [42,43] and can serve as a useful indicator in monitoring therapeutic responses in management of some diseases. Present investigation on the changes in red blood cell count in diabetic rats showed significant reduction compared with the control. This result is consistent with findings of [10]. Normally, red cell count depends on the dynamic balance between the production of new cells and destruction of old ones [44]. This implies that reduction in number of red cells could either result from destabilization of normal erythropoietic process causing a reduced production or increase destruction of the red cell or both [45].

Result showed significant improvement in the red blood cell count of the diabetic group with co-administration of *Terminalia catappa*+aspirin and *Terminalia catappa*+meloxicam compared with diabetic group. The result was similar to that of only aspirin and meloxicam respectively which were also higher than diabetic group. It was observed that the increase in red blood cell count was marginally higher when compared with groups respectively treated with only aspirin and meloxicam. This suggest the possibility of obtaining anti-inflammatory outcome similar to NSAID on red blood cell count by combining the anti-inflammatory drugs with aqueous extract of *Terminalia catappa* though the adverse effect of aspirin and meloxicam were not investigated in this research to ascertain this claim. Furthermore, the significant reduction in packed cell volume (PCV) and haemoglobin (HGB) level in the diabetic group buttressed the findings, this observation is in line with the findings other research [46]. It is reported that decrease in HGB causes decrease deformability of RBC [47] thus increasing haemolysis. Chronic hyperglycemia is known to trigger glycation in several compounds of the erythrocyte including erythrocyte membrane and haemoglobin [48]. Haemoglobin is the most abundant protein in RBC and non-enzymatic glycation of haemoglobin reduces its concentration in diabetes mellitus [49]. The functioning of aspirin is by inhibition of glycation process on haemoglobin thus reducing negative effects on the life span of red blood cells [50]. The contributory effect of hyperglycemia and glycation lead to production of lipid peroxides resulting in RBC hemolysis [51]. The observed results in aspirin and aspirin+extract may activate similar mechanism(s) to reverse the alteration of inhibiting the glycosylation and acetylation processes. But previous studies have suggested amelioration of oxidative stress by extract of *Terminalia catappa* through COX 1 and COX 2 as it is peculiar with phenols.

Further information on the anaemic state of the diabetic rats was obtained from other blood indices. The result of mean corpuscular volume MCV in the diabetic group was reduced compared with control. The result is consistent with the findings of [52,46] in diabetic rats. Mean corpuscular volume is the average size and volume of red blood cell which help in the determination of aetiology of anaemia. Anaemia can be classified based on MCV as macrocytic, normocytic and microcytic depending on the range of the value. The observed reduction of MCV in the diabetic group suggests the presence of microcytic type of anaemia. Research reports has established common causes of microcytic anaemia to include iron deficiency, inflammatory diseases and thalassemia [53], dietary deficiency of vitamin C and gastrointestinal disorders [54]. Research findings has shown that diabetes can contribute to anaemia through reduced absorption of iron, gastrointestinal bleeding and neuropathy [55]. The observed increase in MCV by aspirin, meloxicam and in

combination with *Terminalia catappa* extract indicates correction of the suspected microcytic anaemia. However, there was no investigation on the specific factor(s) influenced by aspirin or meloxicam. Besides the use of MCV on determining the type of anaemia, it is reported to be a potential risk factor of peripheral artery disease and therefore can be used as a predictor of diabetic macrovascular complications [56]. Thus, the restoration of MCV by co-administration of nonsteroidal anti-inflammatory drugs and *Terminalia catappa* similar to aspirin and meloxicam suggest possible amelioration of diabetes related cardiovascular complications with the view of averting the undesirable tendencies associated with aspirin and meloxicam.

The Mean corpuscular haemoglobin (MCH) in the diabetic group was not changed. It is said that MCH can either be reduced or not changed in pernicious and macrocytic anaemia but reduced in microcytic anaemia. This result is contrary to these assertions because the MCH does not follow such pattern in the purported microcytic anaemia as may be observed in this study by the reduced MCV. There were also no changes in MCH levels of the aspirin, meloxicam and *Terminalia catappa* co-administration groups.

On the other hand, the mean corpuscular haemoglobin concentration (MCHC) was observed to increase in diabetic group compared with control. This result is not consistent with the finding of [52] who reported a reduction in MCHC in diabetic rats but agrees with the findings of [57] who reported increased MCHC in human diabetics and report of [58]. The MCHC of diabetic rat treated with anti-inflammatory drugs and in combined drug and *Terminalia catappa* extract showed significant reduction compared with the diabetic group but no significant difference with control. It is known that increased MCHC occurs when red blood cell is fragile or destroyed [58] and in a poor glycemic control diabetic patient [28]. Considering the status of RBC count in this study, the result of MCHC is in line with the earlier observations regarding RBC, HGB and PCV that anaemia was developed and this may be suggestive of red blood cell destruction due to fragility which will requires fragility test for confirmation. Therefore, the reduction in the MCHC following administration of aspirin, meloxicam and their combination with *T. catappa* extract portrays the restoration of the red blood cell membrane integrity.

It has been reported that MCHC and RDW are predictive markers for underlying inflammatory state in diabetes mellitus [59]. Therefore, the observed decrease in MCHC of diabetic treated groups may be attributed to reduced inflammation by anti-inflammatory drug and *T. catappa* extract. The red cell distribution width (RDW-SD) was reduced marginally in diabetic group compared with control but diabetic groups treated with aspirin and meloxicam showed significantly higher RDW-SD than the diabetic group although these were not different from control group. The increase observed in combined drugs and *T. catappa* treated groups were similar to that of the NSAID and was not significant. The benefit of the NSAID may still be achievable with relatively low drug induced complications

From the result of this study the red cell size variance showed marginal reduction in the diabetic group compared with the control. The situation was reversed by the administration of aspirin, meloxicam, and the combined drugs with the extract of *Terminalia catappa*. But there was no difference in the RDW-CV suggesting no significant change in the variation of the cell size.

The reversal of all observed changes in the blood parameters (RBC count, PCV, HGB, MCV) in diabetic group by aspirin, meloxicam and in combination with extract of *Terminalia catappa* indicates that these substances could be effective directly or indirectly in eliminating some risk factors associated with development of anaemia in diabetes mellitus and their associated cardiovascular complications

5. Conclusion

The blood cell parameters of diabetic rats administered with nonsteroidal anti-inflammatory drugs; aspirin and meloxicam were observed to improve significantly. The results showed significant elevation of RBC count, PCV, HGB and MCV confirming existence of strong relationship between anaemia in diabetes and inflammation. In view of the current controversy on the use of aspirin in treatment of cardiovascular complication in diabetes, co-administration of anti-inflammatory drugs with *Terminalia catappa* can provide an avenue to benefit from the usefulness of aspirin and meloxicam while preventing the reported negative effect

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experimental protocol received full ethical approval from Faculty Animal Research Ethics Committee-Faculty of Basic Medical Sciences (FAREC-FBMS) with approval number 021PY30417.

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