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SYNERGISTIC EFFECT OF COMBINED MULTIPLE PLANTS EXTRACT ON SOME BLOOD CELL INDICES OF DIABETIC RATS

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ABSTRACT

This study evaluated the impact of combined therapy of multiple plants extract of Cnestis ferruginea, Xylopia aethiopica, Palisota hirsuta, Scleria sp., Napoleona imperialis, Dialium guineense, Combretum racemosun, Heterotis rotundifolia leaves, stem of Sphenocentrum jollynum stem, and root of Uvaria chamae on blood cell indices of diabetics. Female Wistar rats of 40 – 50 g and fifty-four (54) in number were used for this study. They were assembled into 6 groups of 9 rats each. Group I served as the normal control (NC) while the remaining five groups were induced with diabetes type 2 using high-fat diet (HFD) for 8 weeks and asingle dose intraperitoneal injection of streptozotocin at 35 mg/kg body weight. Group II was the diabetic control (DC) while the other groups (III, IV, V & VI) were orally administered 7.2 mg/kg metformin and the cocktail extract at three different concentrations of 500 mg/kg, 250 mg/kg, and 100 mg/kg respectively. Diabetes was established after HFD and STZ administration. Blood cell indices such as platelets, red blood cells (RBCs), white blood cells (WBCs) and differentials were evaluated after twelve weeks of treatment using a hematology auto-analyzer. Results obtained showed that the diabetic control group recorded lower platelets, RBC, WBC, neutrophil and eosinophil as well as higher lymphocyte and monocyte when compared to the groups administered the cocktail extract and other experimental groups. This study revealed that the combined therapy of the multiple plants extract has positive effect on blood cell indices and can be adopted as a blood cell boosting agent.

Keywords: Aju Mbaise, Combined Multiple Plant Extract, Diabetes, Haematology, and Streptozotocin.

INTRODUCTION

Diabetes mellitus type 2 (DMT2) and its complications is still generally considered as an expanding global health challenge with very high mortality rate especially in poor countries where accessibility to quality healthcare is rare. This chronic metabolic disorder has become prevalent with its continuous rapid increase globally. According to Cho *et al.* (2018), DMT2 is fast becoming an epidemic in some countries of the world with the number of diabetics expected to

increase to 693 million by the year 2045. This devastating postulate can be associated to increase in ageing population, thereby adding to the already existing burden for healthcare providers, especially in poorly developed countries. Corrao *et al.* (2021), posited that diabetics are highly susceptible to complications emanating especially from viral infections, though dependent on the degree of viral load, host immune response, age of the patient, and presence of comorbidities. The pathologic hallmark of DM is linked to various microvascular and macrovascular complications (Sun *et al.*, 2022) such as neuropathy, nephropathy, retinopathy, foot ulcer disease (FUD), hearing impairment, and Alzheimer's disease (Onyeji, 2022). According to Unnikrishnan and Misra (2021), the global undiagnosed diabetics is estimated at about 193 million, predisposing them to several long-term complications of untreated chronic hyperglycemia. In diabetes, haematological changes are directly associated with endothelial dysfunction and inflammation. According to Ebrahim *et al.* (2022), DM can be associated to glucose intolerance, hyperinsulinemia, dyslipidemia, insulin resistance syndrome, inflammation, thrombophilia, dysglycemia, oxidative stress, inflammation, endothelial dysfunction, haemostatic and haematological abnormalities, and the generation of atherogenic lipoproteins. Persons with inadequate diabetes management may have a significant alteration in metabolic, cellular, and haematological disturbances that leads to vascular complications (Farooqui *et al.*, 2019). Ebrahim *et al.* (2022), stated that haematological alterations such as change in the function, structure, and metabolism of RBCs, WBCs, platelet count and its indices, and haemostatic parameters are the common abnormalities seen in DMT2 patients. According to Umeji *et al.* (2019), anemia remains one of the commonest haematological abnormalities usually seen in DM patients, occurring earlier and especially in patients with diabetic nephropathic complications. According to Parwani and Mandal (2023), prolonged hyperglycemia may lead to increased production of reactive oxygen species (ROS) and the formation of advanced glycation end products (AGEs) which are directly associated with endothelial dysfunction and haematological alterations. The increased generation of oxidative stress may cause tissue damage and haematological alterations such as RBC dysfunction, platelet hyper activation, and endothelial dysfunction (Vona *et al.*, 2021). According to Abdel-Moneim *et al.* (2020), oxidative stress results in RBC dysfunction, platelet destruction, and tissue injury which may affect the function of blood cells and the haemostatic parameters that may lead to various complications. According to Shin *et al.* (2020), insulin resistance is also associated with endothelial dysfunction, increased plasma level of inflammatory markers, increased WBC count, and platelet hyper activation that may trigger and accelerate vascular complications in DMT2 patients. DM is characterized by high level of glucose in the blood, emanating from insufficient concentration and/or activity of insulin due to declined insulin production, and eventual pancreatic beta-cell failure, thereby inhibiting glucose transport into the liver, muscle cells, and fat cells. Thus, insulin resistance and impaired insulin secretion remain the core deficiencies in DMT2; though other pathophysiological abnormalities, environmental factors and genetic factors contribute to the multiple pathophysiological disorders that are responsible for impaired glucose balance which contributes to dysregulation of glucose metabolism. According to Artasensi *et al.* (2020), these multiple pathogenetic disorders of DMT2 predicts that combination therapy of multiple antidiabetic agents will be required to maintain normal glycaemia. Currently, adoption of healthy diet, physical exercise, pharmacological therapy and/or insulin are used to maintain normal blood glucose level as near as possible and to delay or possibly prevent the progression of diabetes-related complications as there is no cure for diabetes yet. The existing

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hypoglycemic agents (antidiabetic drugs) includes biguanides, meglitinides, sulfonylureas, alpha-glucosidase inhibitors, and thiazolidinediones. According to Nnadiukwu *et al.* (2023), these antidiabetic drugs effectively maintained normoglycaemia when administered appropriately, but also are tiresome, expensive, alongside its adverse effects. Considering the aforementioned factors, there is need for the adoption of plant and its product for the treatment and/or control of diseases especially DMT2. According to Verma *et al.* (2018), DM can be dealt with plants via mechanisms such as stimulation of insulin synthesis and acting like insulin, regeneration of damaged pancreatic beta cells, improvement of insulin sensitivity, altering the activities of enzymes that catalyze glucose metabolism, and by inhibition of glucose absorption. This study adopted the combination therapy of multiple plants extract for the treatment of DMT2. The combined form of these selected plants (*Sphenocentrum jollynum, Cnestis ferruginea, Xylopia aethiopica, Uvaria chamae, Palisota hirsuta, Scleria sp., Napoleona sp., Dialium guineense, Combretum racemosun, and Heterotis rotundifolia*) is commonly referred to as 'Aju Mbaise' by the people of South-Eastern Nigeria (Nnadiukwu *et al.,* 2019). This cocktail effectively detoxifies, sanitizes the womb, as well as reduces stomach to its original size and shape in good time when administered to women after childbirth. It also boosts the mineral and vitamin levels in lactating mothers (Ezejindu and Iro, 2017). These plants have shown their individual therapeutic effects against so many diseases. *Sphenocentrum jollynum* possesses anti-diabetic (Alese *et al.,* 2014), anti-inflammatory and anti-allergic (Olorunnisola *et al.,* 2017), anti-bacterial (Koleosho *et al.,* 2013), antioxidant and anti-malaria (Olorunnisola and Afolayan, 2013) effects. Adisa *et al.* (2014), reported the in vivo hypoglycaemic activity of methanol leaf concentrates of *C. ferruginea* in STZ-induced diabetic rodents. Hypoglycaemic and hypocholesterolemic activities of *C. ferruginea* leaves was also reported by Ndip *et al.* (2013). According to Bhatt *et al.* (2016), *C. ferruginea* contains isoflavone, coumarin, and anthocyanins which have been reported to possess antidiabetic effect. According to Nsuadi *et al.* (2012), *C. racemosun* possesses anti-inflammatory, vasorelaxant, and trypanocidal properties. *D. guineense* was reported by Nijveldt *et al.* (2001), to have shown antimalaria, anti-inflammatory, and antioxidant properties. According to Etekpo *et al.* (2018), *H. rotundifolia* contains phenolic and flavonoic compounds that are responsible for its antioxidant activity. Esimone *et al.* (2005), reported that methanolic extract of *N. imperialis* showed a fast wound healing property. This effect alongside bactericidal action could be attributed to the presence of tannins, glycosides, saponins and proteins (Chah *et al.,* 2006). Boakye-Gyasi *et al.* (2011), reported the anti-inflammatory, antipyretic, and anti-oxidant effects of *Palisota hirsuta*. Okwuosa *et al.* (2012), reported the hypoglycaemic, antifungal, bacteriostatic and antimalaria properties of *Uvaria chamae*. The therapeutic actions of these individual plants are attributed to the bioactive compounds present in them. Thus, the collective bioactive compounds of these plants will have an incredible wide therapeutic effect. The combination of more than one class of drug constituent is commonly used in medicine. According to Vaou *et al*. (2022), a particular strategy does not constantly enrich its particular pharmacological effect; thus, combinations comprising two or more components can afford additive, synergistic, or antagonistic effects. Also the use of combination therapy of multiple plants extract for medicinal purpose especially in traditional healing systems is a global practice aimed at achieving desired therapeutic goal. These plants are believed to contain many bioactive compounds which include phytochemicals, proximate nutrients, vitamins and minerals. In a combined therapy, each bioactive compound exerts a synergistic or an additive effect, thus improving therapeutic efficacy while possibly reducing dose and toxicity. According to

Mason and Routledge (2005), combination therapy has been applied in traditional herbal medicines since the emergence of therapeutics. According to Chou (2010), synergistic therapeutic effect is aimed at dose and toxicity reduction and further minimizing the emergence of drug resistance. Koizumi and Iwami (2014), reported several scientific investigations that have indicated the efficacy of two or more different therapeutic agents used in combination that either induced synergistic or antagonistic interactions. Recall, high blood glucose and its metabolic syndrome can be related to the alteration of the different haematological parameters such as the morphology, size, and physiological functions of RBCs, WBCs, and platelets. This alteration can be attributed to the activities of reactive oxygen species (ROS) that are predominant in diabetes and its complications. According to Uko *et al*. (2013), patients with DM show a significant instability in various haematological parameters. According to Szydełko *et al.* (2018), hyperglycemia, dyslipidemia, insulin resistance, and oxidative stress could stimulate the production of pro-inflammatory cytokines, activation of inflammatory signaling pathways, and recruitment of immune cells that can contribute to the elevated level of leukocytes and its sub-population. Bhattacharjee *et al.* (2016), also posited that hyperglycemia, insulin resistance, and insulin deficiency contribute to increased platelet reactivity through direct effects via promoting glycation of platelet proteins leading to morphological and functional alteration of platelet indices in DMT2 condition. Furthermore, Otieno *et al.* (2008), stated that multi-plant extracts were more superior over single plant extracts and could be developed into more potent antibiotics against resistant pathogens and advocated for the preference and effectiveness of mixed extracts by traditional healers in management of opportunistic infections. The study was based on the unavailability of prior laboratory tests to diagnose the exact causes or agents of ailments. Thus, the use of multi-plant extract regimens expand the confidence level that, most of the causes of the ailment may be managed with any one of the mixture's ingredients. Considering the prediction by Artasensi *et al.* (2020), for the adoption of combination therapy of multiple antidiabetic agents, the limited studies that investigated the alterations of haematological parameters in DMT2, and the rising interest in the use of plant and its product for therapeutic purpose, this study investigated the impact of this cocktail of multiple plants on some haematological indices in diabetic female Wistar rats.

MATERIALS AND METHODS

Reagents

All the biochemical reagents, chemicals and materials used in this research work were of standard analytical grade. Streptozotocin (STZ) was acquired from Sigma Chemicals Co. St. Louis, MO, USA, and Metformin from Merck Serono, Milano, Italy.

Collection and Identification of Plant Samples

Fresh samples of the individual plants were collected at Obodo Ujichi, Ahiazu and Amuzi, Ahiara Towns, in Ahiazu Mbaise L.G.A, of Imo State, Nigeria. The plant samples were identified as *Cnestis ferruginea*, *Xylopia aethiopica*, *Uvaria chamae*, *Palisota hirsuta*, *Scleria sp*., *Napoleona imperialis*, *Dialium guineense*, *Combretum racemosun*, and *Heterotis rotundifolia* at the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. The fresh plants parts after collection were air dried, cut into pieces and pulverised before extraction using maceration method. The resultant extract was weighed and stored in clean bottles until usage.

Preparation of high fat diet (HFD)

The HFD was constituted in accordance to the method of Liu (2018), using standard laboratory chow (Top feed) growers mash, lard and sucrose at 3:1:1 ratio respectively.

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Experimental Animals

A number of fifty-four (54) female Wistar rats were used for this study. This gender was preferred as they mostly consume this herbal cocktail. The rats were procured from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. At the time of procurement, the rats weighs between 40 – 50 g. They were housed in the animal house of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria, and were left for seven days to adapt to the experimental conditions during which they were given normal chow (Top feeds-grower's mash) and clean water. The animals were handled in line with the global guiding principles for the care and use of animals for scientific purposes. The Research work was approved by the Research Ethics Committee of the University of Port Harcourt with authorization number: UPH/CEREMAD/ REC/MM64/003.

Induction of Type 2 DM

DMT2 was induced to the experimental rats (Groups II - VI) with a single dose intraperitoneal injection of 35 $mgkg^{-1}$ body weight (b.w) of STZ prepared with normal saline. Fasting blood glucose was checked to ascertain DM after 7 days of STZ administration. This was followed by oral administration of metformin and the extracts which was done daily for a period of twelve (12) weeks. At every four weeks interval, three animals from each group were fasted overnight, anaesthetized, sacrificed, and blood samples collected with EDTA bottles for haematologicalexamination.

Experimental Design

The experimental animals were gathered into six groups of nine animals each. The diabetic animals in group II remained untreated while those in groups III to VI were treated with metformin 7.2 $mgkg^{-1}$ b.w, and three different doses of the cocktail extract respectively, as shown in the table below;

Table 1: Groupings of the Experimental animals

Groups	Code	Treatment
I.	**Normal Control**	The animals in this group were not induced with diabetes but were given distilled water and normal feed throughout the experiment.
II.	**Diabetic Control**	The animals in this group were made diabetic, received water and normal feed but remained untreated throughout the experiment.
III.	**Metformin Treated**	Diabetic animals treated with 7.2 $mgkg^{-1}$b.w metformin
iv.	**500 mg/kg Extract**	Diabetic animals treated with 500 $mgkg^{-1}$b.w of the combined multiple plants extract(CMPE)
v.	**250 mg/kg Extract**	Diabetic animals treated with 250 $mgkg^{-1}$b.w of the CMPE
vi.	**100 mg/kg Extract**	Diabetic animals treated with 100 $mgkg^{-1}$b.w of the CMPE

Determination of Haematological Parameters

Haematological indices such as red blood cell (RBC), white blood cell (WBC), platelet count, neutrophils (Neu.), lymphocytes (Lym.), eosinophils (Eos.), and monocytes (Mon.), were determined with haematology auto-analyzer. According to the procedure, the blood samples were properly mixed and

homogenized by inverting 8 – 10 times following the safety procedures and specimen handling procedures, after which it was introduced into the auto-analyzer. The parameters to be analyzed were selected and the machine was allowed to run for 2-3 minutes after which the results was displayed on the screen and then printed.

Statistical analysis

Result values were presented as Mean ± standard error of mean (SEM), while Tukey Test of one way ANOVA was applied to test for significant differences between treatment groups using the Statistical Package for the Social Sciences (SPSS) (version 25.0). The results were considered significant at ($p<0.05$).

RESULTS

The results of the haematological analysis which includes red blood cell, white blood cell, platelet count, neutrophils, lymphocytes, eosinophils, and monocytes were presented in Tables 2 – 8 respectively.

Effect of the combined multiple plants extract (CMPE) on Haematological indices of High fat diet and Streptozotocine (HFD/STZ) induced diabetic Wistar rats

From the result presented in Table 2, the diabetic control (DC) group has RBC count that is lower than that of the other experimental groups throughout the experimental period. The normal control (NC) group recorded the highest RBC in week 12, while the group administered 500mgkg^{-1} b.w. CMPE recorded the highest RBC in weeks 4 and 8. The only significant difference was recorded in week 8 when the group administered 500 mgkg^{-1} b.w. CMPE was compared to the DC group. From the result presented in Table 3, the diabetic control (DC) recorded WBC count that is lower than that of the other experimental groups throughout the experimental period. The NC group recorded the highest WBC in week 12, while the groups administered 100 mgkg^{-1} and 250 mgkg^{-1} b.w. CMPE recorded the highest WBC in weeks 4 and 8 respectively. The CMPE treated groups also recorded higher WBC than the metformin treated group, though there was no significance difference in the WBC count among the experimental groups within the experimental period. In Table 4, the DC group has lower platelet count when correlated with the other experimental groups within the experimental period. The NC group recorded the highest platelet count in weeks 4 and 8 respectively, while the metformin treated group recorded the highest platelet count in week 12. In Table 5, the DC group recorded a significantly lower neutrophil count when compared with the NC in week 4 as well as the groups treated with metformin and 500 mgkg^{-1} b.w. CPME in week 8. Meanwhile, the group administered 100 mgkg^{-1} b.w. CMPE recorded the lowest neutrophil count in week 12. Also the NC, metformin treated and the group administered 500 mgkg^{-1} b.w. CMPE recorded the highest neutrophil count in weeks 4, 8, and 12 respectively. From the result presented in Table 6, the DC group recorded a lymphocyte count that was significantly higher than groups treated with metformin and 500 mgkg^{-1} b.w. CMPE in week 8. Meanwhile, the group treated with 100 mgkg^{-1} b.w. CMPE recorded the highest lymphocyte count in week 12. Also the NC, metformin treated and the group administered 500 mgkg^{-1} b.w. CMPE recorded the lowest neutrophil count in weeks 4, 8, and 12 respectively. In Table 7, the group administered 100mgkg^{-1} b.w. CMPE recorded significant higher ($p<0.05$) eosinophil count than the rest of the experimental groups in weeks 4 and 8 respectively. No significant difference among the groups was seen in week 12 though the group administered 100 mgkg^{-1} b.w. CMPE recorded the highest eosinophil count while the DC, metformin treated and the group treated with 500mgkg^{-1} b. w. CMPE recorded the least eosinophil count in weeks 4, 8 and 12 respectively. From the result presented in Table 8, the group that received 100mgkg^{-1} b. w. CMPE recorded monocyte count that was

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significantly lower than that of the other experimental groups in weeks 4 and 8 respectively. Meanwhile, the DC and metformin treated groups recorded the highest monocyte count in weeks 4 and 8 respectively.

Table 2: Effect of the CMPE on RBC count of HFD/STZ-induced diabetic Wistar rats

	RBC ($x10^6/mm^3$)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	6.73±0.29	6.50±0.17^{ab}	6.70±0.26
DC	6.10±0.12	6.13±0.09^{a}	5.83±0.32
Metformin	6.67±0.22	6.57±0.12^{ab}	6.33±0.12
500 mg Extract	6.83±0.61	6.97±0.50^{b}	6.00±0.20
250 mg Extract	6.27±0.39	6.63±0.09^{ab}	6.07±0.57
100 mg Extract	6.57±0.37	6.27±0.09^{ab}	5.97±0.20

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ b. w. of the CMPE; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ b. w. of the CMPE; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ b. w. of the CMPE.

Table 3: Effect of the CMPE on WBC count in HFD/STZ-induced diabetic Wistar rats

	WBC ($x10^3/mm^3$)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	11.10±0.67	10.50±0.51	12.37±0.45
DC	8.87±1.62	9.47±1.18	9.17±1.37
Metformin	10.13±1.94	9.60±1.45	11.70±1.12
500mg Extract	11.07±0.92	10.77±1.19	9.57±1.88
250mg Extract	11.07±2.63	11.10±0.81	11.83±2.26
100mg Extract	11.43±0.67	10.87±0.41	10.03±1.01

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ b. w. of the CMPE; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ b. w. of the CMPE; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ b. w. of the CMPE.

Table 4: Effect of the CMPE on Platelets count in HFD/STZ-induced diabetic Wistar rats

	Platelet ($x10^3/ml$)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	335.00±31.22	346.67±20.28	308.33±16.91
DC	254.00±10.69	266.33±22.92	218.00±15.95
Metformin	282.67±5.81	292.00±14.42	316.00±12.06
500mg Extract	267.33±3.93	286.67±16.91	261.67±49.10
250mg Extract	273.00±12.66	301.00±10.02	267.33±44.41
100mg Extract	255.67±25.41	293.00±23.63	269.67±25.31

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control;**Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ b. w. of the CMPE; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ b. w. of the CMPE; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ b. w. of the CMPE.

Table 5: Effect of the CMPE on Neutrophil in HFD/STZ-induced diabetic Wistar rats

	Neutrophil (%)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	26.67±1.67^{b}	25.00±0.00^{ab}	26.67±1.67
DC	19.33±0.67^{a}	20.67±1.76^{a}	22.67±3.71
Metformin	25.33±2.91^{ab}	27.33±1.33^{b}	25.67±3.48
500mg Extract	25.00±1.15^{ab}	26.67±0.88^{b}	27.00±0.58
250mg Extract	25.00±0.58^{ab}	24.67±0.33^{ab}	25.33±0.67
100mg Extract	23.67±0.88^{ab}	24.67±0.33^{ab}	21.67±3.76

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 mgkg^{-1} b. w. of the CMPE; **250 mg Extract** = Treated with 250 mgkg^{-1} b. w. of the CMPE; **100 mg Extract** = Treated with 100 mgkg^{-1} b. w. of the CMPE.

Table 6: Effect of the CMPE on Lymphocyte in HFD/STZ-induced diabetic Wistar rats

	Lymphocyte (%)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	63.33±1.67	65.00±.00^{ab}	63.33±1.67
DC	70.67±0.67	69.33±1.76^{b}	67.33±3.71
Metformin	64.67±2.91	62.67±1.33^{a}	64.33±3.48
500mg Extract	65.00±1.15	63.33±0.88^{a}	63.00±0.58
250mg Extract	65.00±0.58	65.33±0.33^{ab}	64.67±0.67
100mg Extract	68.67±3.18	65.33±0.33^{ab}	68.33±3.76

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 mgkg^{-1} b. w. of the CMPE; **250 mg Extract** = Treated with 250 mgkg^{-1} b. w. of the CMPE; **100 mg Extract** = Treated with 100 mgkg^{-1} b. w. of the CMPE.

Table 7: Effect of the CMPE on Eosinophil in HFD/STZ-induced diabetic Wistar rats

	Eosinophil (%)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	3.00±0.00^{a}	2.67±0.33^{a}	3.33±0.33
DC	2.33±0.33^{a}	2.67±0.33^{a}	3.00±0.58
Metformin	2.67±0.33^{a}	2.33±0.33^{a}	3.00±0.58
500mg Extract	3.67±0.33^{a}	3.00±0.00^{a}	3.00±0.58
250mg Extract	3.33±0.33^{a}	3.67±0.33^{a}	3.67±0.33
100mg Extract	6.00±0.58^{b}	6.33±0.33^{b}	4.33±1.45

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 mgkg^{-1} b. w. of the CMPE; **250 mg Extract** = Treated with 250 mgkg^{-1} b. w. of the CMPE; **100 mg Extract** = Treated with 100 mgkg^{-1} b. w. of the CMPE.

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Table 8: Effect of the CMPE on Monocyte in HFD/STZ-induced diabetic Wistar rats

	Monocyte (%)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	7.00±0.00^{b}	7.33±0.33^{b}	6.67±0.33
DC	7.66±0.33^{b}	7.33±0.33^{b}	7.00±0.58
Metformin	7.33±0.33^{b}	7.67±0.33^{b}	7.00±0.58
500mg Extract	6.33±0.33^{b}	7.00±0.00^{b}	7.00±0.58
250mg Extract	6.67±0.33^{b}	6.33±0.33^{b}	6.33±0.33
100mg Extract	4.00±0.58^{a}	3.67±0.33^{a}	5.67±1.45

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not. **Key: NC=** Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 mgkg^{-1} b. w. of the CMPE; **250 mg Extract** = Treated with 250 mgkg^{-1} b. w. of the CMPE; **100 mg Extract** = Treated with 100 mgkg^{-1} b. w. of the CMPE.

DISCUSSION

This study considered the synergistic effect of the combined multiple plants extract (CMPE) comprising of *S. jollynum, C. ferruginea, X. aethiopica, U. chamae, P. hirsuta, Scleria sp., Napoleona sp., D. guineense, C. racemosun, and H. rotundifolia* collectively referred to as Aju Mbaise in South-East, Nigeria on some haematological indices in diabetic rats. The results presented above (Tables 2-8) revealed that the diabetic control group recorded lower RBC, WBC, platelets, neutrophil and eosinophil as well as higher lymphocyte and monocyte count when compared to the other experimental groups which includes the groups treated with the CMPE throughout the experimental period. The result also indicated that CMPE administration enhanced the RBC, neutrophil and eosinophil counts and as well reduced the lymphocyte and monocyte counts. The WBC and platelet counts were not significantly influenced by CMPE administration. This is in consonant with the report of Umeji *et al.* (2019), that revealed significant lower RBC, platelet, and eosinophil counts in DMT2 patients when compared to the healthy control group, and in the same vein contradicts Umeji *et al.* (2019), report that revealed significant higher WBC, and neutrophil counts and low monocyte count in DMT2 patients when compared to the healthy control group. Twig *et al.* (2013), also reported that for every increase in within the normal range of the WBC count, the risk for diabetes increases by 7.6%. The result of the current study is in total compliance with Tihić-Kapidžić *et al.* (2021), who reported significant lower RBC, WBC and platelet count in diabetic children when compared to the healthy control group. Ebrahim *et al.* (2022), reported significant difference in the monocyte, basophil, RBC, hematocrit, MCV, MCH, RDW-SD, MPV, PDW, PLC-R, and platelet counts between DMT2 patients and healthy control group, though FBG was statistically negatively correlated with some haematological parameters (RBC, Hgb, Hct, MCV, MCH and RDW-SD) and statistically positively correlated with the other haematological parameters (WBC, neutrophil, monocyte, basophil, RDW-CV, PDW, MPV, PLC-R, and plateletcrit). The possible explanation to this outcome might be associated to persistent hyperglycemia which has a direct relationship with the non-enzymatic glycation of various proteins and the increased expression of pro-inflammatory cytokines such as IL-6, and TNF- α in blood circulation. Elevation of pro-inflammatory cytokines are key in establishing insulin resistance as well as the changes in the sensitivity of progenitors to erythropoietin (erythroid growth factor). According to Bhatt *et al.* (2020), inflammation may stimulate apoptosis of immature erythrocytes leading to decreased circulating erythrocytes consequently causing a reduction in circulating haemoglobin otherwise known as

anemia. According to Mahmoud (2013), anemia may result from diminished erythropoietin production by failing kidney and increased non enzymatic glycosylation of red blood cell membrane proteins. Anemia prevalence in mostly female DMT2 patients has been reported by Ebrahim *et al.* (2022). Al-Khoury *et al.* (2006), posited that DM is one of the common causes of anemia. Idris *et al.* (2018), posited that DMT2 patients who had diabetic complications and longer duration of diabetes were significantly associated with anemia. Forte *et al.* (2011), mentioned erythropoietin deficiency, iron deficiency, decreased lifespan of red blood cells, chronic blood loss, chronic inflammation, oxidative stress, nutritional deficiency, and chronic suppression of erythropoiesis as several factors responsible for the development of anemia in diabetic condition. According to Muhammad *et al.* (2020), development of anemia by DMT2 patients can be triggered by longer exposure to hyperglycemia which is significantly associated with the generation of reactive oxygen species (ROS) that may be related to multiple organ damage. Wang *et al.* (2021), affirmed that RBCs metabolism in diabetic patients may be altered by multiple risk factors such as hyperglycemia, hyperosmolarity, oxidative stress, inflammation, and lipid metabolic disorder, thus, leading to increased aggregation, reduced cell deformability, and membrane fluidity. Consequently, limiting the survival rate, morphology, size, and physiological functions of erythrocytes, thereby aggravates diabetic complications. The CMPE showed a promising effect against anemia. The positive activities of the CMPE on the assayed haematological parameters might be attributed to the collective phytochemicals present in the individual plants that constitute the multi-plant combination. Nnadiukwu *et al*. (2019), reported that the CMPE applied in this study is rich in phytochemicals such as phenols, flavonoids, tannins, alkaloids, saponins, steroids, glycoside, and terpenoids. Nnadiukwu *et al*. (2023), also reported the antidiabetic effect of this CMPE commonly referred to as Aju Mbaise. According to Nnadiukwu *et al*. (2020), CMPE of Aju Mbaise contains appreciable amount of minerals (arsenic, aluminum, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, silver, sodium, and zinc), vitamins (A, B_1, B_2, B_3, B_6, B_{12}, C, D, and K), and nutritional components like carbohydrate, protein, moisture and fat; with negligible amount of crude fibre and ash. Nutrients such as carbohydrate, proteins, lipids, fiber, minerals and vitamins are known to regulate tissue functioning, and effective metabolic reactions in the body. It can be stated that utilization of this CMPE can help fight against mineral and vitamin deficiencies since it contains a wide range of mineral elements and vitamins which are equivalent to those found in diets. According to Burk *et al.* (2006), pyridoxine (Vit. B_6) is essential for protein digestion, growth, synthesis of phospholipids and sphingolipids, while cobalamin (Vit. B_{12}) is essential for haemopoiesis, and normal functionality of the nervous system. CMPE is considered for aiding transport and uptake of non-heme iron at the mucosa, and stimulation of cortisol synthesis due to its vitamin C content. According to Riccioni *et al.* (2007), ascorbic acid expressed its efficacy in reducing sorbitol secondary diabetes and lipid peroxides generated by oxidative stress and free radicals. Meanwhile, its deficiency leads to fragility of blood vessels, gum rot and scurvy. Nnadiukwu *et al*. (2020), also reaffirmed the vital roles of minerals which includes normal growth, muscles activities and skeletal development, cellular activities and oxygen transport, chemical reaction in the body and intestinal absorption, fluid balance and nerve transmission. The impact of combination therapy of multiple plants cannot be overemphasized. According to Donkor *et al*. (2023), synergy might have resulted from the targeting of multiple pathways, which may include substrates,

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enzymes, metabolites, ion channels, ribosomes, and signal cascades.

CONCLUSION

The result of the key haematological parameters recorded in this study revealed that DMT2 can trigger significant alteration in haematological indices. This study revealed that DMT2 can lead to low RBC, WBC, platelet, neutrophil, and eosinophil as well as high lymphocyte and monocyte counts. The study furthermore revealed the efficacy of the combined multiple plant extracts (CMPE) in the management and/or control of DMT2. The synergistic attributes exhibited by the CMPE interactions in this work could be attributed to the different bioactive compounds in the individual plants. This action might be linked to the targeting of multiple pathways. The use of CMPE has shown to have a broad therapeutic model, considering its multiple targets, links, and approaches. Thus, could be applied for the development of new effective antidiabetic agents. Finally, haematological makers should not be neglected in the early diagnosis and proper management of DM and its related complications.

Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

Nnadiukwu, C. U. and Nnadiukwu, T. A. designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.

Nnadiukwu, T. A. managed the analyses of the study.

Nnadiukwu, C. U., Ajuru, G. and Ogono, T. G. managed the literature searches.

All authors read and approved the final manuscript.

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