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Investigations of preventive and ameliorative effects of *Cucurbita maxima* leaf supplemented diets on haematological parameters in STZ-induced diabetic albino rats

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Abstract

No effective treatment for diabetes has been found in medical science thus far. Anti-diabetic medications treat diabetes mellitus symptoms but not the underlying illness process. Some synthetic medications with anti-diabetic properties are already available for oral administration; they include synthetic hypoglycemia agents like sulfonylureas families. This study investigated the preventive and ameliorative effects of *Cucurbita maxima* leaf supplemented diets on haematological parameters in stz-induced diabetic albino rats. Sixty rats (60) were randomly distributed into 6(six) groups of five animals each (n= 5) for both preventive and ameliorative studies respectively. For preventive treatments, rats were supplemented with 5%, 10%, 15% and 20% *Cucurbita maxima* leaf for 28 days and diabetic was induced in week 4. Whereas, for the ameliorative treatments, diabetes was induced in week 0 and rats were supplemented for 28 days. The animals were sacrificed at the end of the experimental period and blood sample was collected for haematological evaluation. Consequently, the significant ($p < 0.05$) decrease in the levels of RBC, Hb and PCV observed in diabetic animals was drastically ($p < 0.05$) increased in 10%, 15% and 20% supplemented groups in both preventive and ameliorative trials compared to STZ-induced diabetic control group. Similarly, the levels of MCH, MCV and MCHC in 5%, 10%, 15% and 20% groups rise significantly ($p < 0.05$) compared to STZ-induced diabetic control in the both experiments. Furthermore, the levels of WBC was significantly ($p < 0.05$) increased after supplementation with 10%, 15% and 20% of *C. maxima* leaf in the preventive and ameliorative experiments as compared to the STZ-induced diabetic control but was not significant at $p < 0.05$ when compared with the normal control. The results obtained through these investigations pointed to both preventive and ameliorative haematinic and erythropoietic potentials of *Cucurbita maxima* leaf in a diabetic state.

Keywords: *Cucurbita maxima* leaf; Red blood cell; White blood cell; haematological; Preventive; Ameliorative

1. Introduction

Chronic hyperglycemia, changes in carbohydrate, lipid, and protein metabolism, and problems in the action of insulin secretion characterise diabetes mellitus (DM), a severe metabolic illness with numerous aetiologies. Lupascu *et al.* (2019) identify it as the most prevalent illness affecting persons of all ages. Several subtypes of diabetes exist. Only around 5-10% of people with diabetes have type 1. Chronic hyperglycemia and dysfunction in insulin synthesis in cells of the islets of Langerhans in the pancreas are hallmarks of the disease. As a result, they bring about insulin insufficiency. Nearly majority instances of diabetes are of the type 2 kind. Diabetes mellitus type 2 is characterised by insulin resistance and relative insulin insufficiency, and it is often associated by excess body fat (Lin *et al.,* 2018). No effective treatment for diabetes has been found in medical science thus far. Anti-diabetic medications treat diabetes mellitus symptoms but not the underlying illness process. Some synthetic medications with anti-diabetic properties are already

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available for oral administration; they include synthetic hypoglycemia agents like sulfonylureas families. Patients with DM may not respond favourably to therapy because of the many adverse effects associated with therapeutic support, including but not limited to weight gain, hypoglycemia, gastrointestinal problems, liver and kidney damage, and hypersensitivity responses (Al-Attar *et al*., 2019).

Cucurbita maxima (pumpkin) is one of the underutilized crops which belong to the family, Cucurbitaceae. Its existence is presently being threatened due to neglect in Nigeria. Pumpkin is cultivated in Nigeria in a subsistence level with virtually no commercial importance. Pumpkin is a vine crop and, it plays an important role in the traditional setting as a cover crop and weed control agent (Delahaut and Newenhouse 2006). In Nigeria, it is a traditional vegetable crop, grown mainly for its' leaves, fruits, and seeds and, consumed either by boiling the leaves and fruits, or by roasting or baking the seeds (Facciola 1990). The leaves, fruits, flowers and seeds are health promoting food. Different parts of the plant have been used as medicine in some developed world. The leaves are haematinic, analgesic, and also used externally for treating burns. Traditionally, the pulp is used to relieve intestinal inflammation or enteritis, dyspepsia and stomach disorders (Sentu and Debjani 2007). Pumpkin fruit is an excellent source of vitamin A which the body needs for proper growth, healthy eyes and protection from diseases. It is also rich in vitamin C, vitamin E, lycopene and dietary fibre (Pratt and Matthews, 2003).

Pumpkin seeds contain many valuable functional components and have been traditionally used for herbal, therapeutic as well as clinical application for safe deworming and diuretic agents, and the seed oil as a nervine tonic (Mitra *et al.,* 2009). Pumpkin seed oil has a strong antioxidant property, and has been recognized for several health benefits such as prevention of the growth and reduction of the size of prostate, reduction of bladder and urethral pressure and improving bladder compliance, alleviation of diabetes by promoting hypoglycemic activity, and lowering level of gastric, breast, lung, and colorectal cancer (Sarvesh, 2012). This study investigated the preventive and ameliorative effects of *Cucurbita maxima* leaf supplemented diets on haematological parameters in stz-induced diabetic albino rats.

2. Materials and methods

2.1. Sample Collection and Preparation

Fresh leaves of *Cucurbita maxima* will be collected from Ejule in ofu LGA of kogi State and taken to a standard Herbarium, for identification, authentication and a voucher will be deposited, it will then be rinsed in clean water, dried at room temperature and ground to powder form, which will then be kept in an air tight container away from sunlight till needed

2.2. Experimental diet

Grower mash feed produced by UAC Company was procured from Jos, Plateau State. The feed served as the normal diet and was also used to mix the powdered leaves of *Cucurbita maxima* to formulate dietary inclusion of (5% and 10%, 15% and 20% w/w) for the experimental group. As described by (Onuche and Abu, 2021).

2.3. Experimental animals/ grouping

Sixty (60) healthy male albino rats of the wistar strain weighing 80-100 g were procured from the animal house, National Veterinary Research Institute (NVRI) Vom, Plateau state, Nigeria. All the animals were maintained under standard laboratory conditions (24 ± 2 ^{o}C, 12/12 h light-dark cycle), fed with a standard pellet diet, and allowed free access to water *ad libitium* during 28 days of the experimental period. Before starting the experiment, all the animals were allowed to acclimatize for laboratory conditions for two weeks. At the end of the acclimatization period, all animals were weighed and randomly distributed into 6(six) groups of five animals each (n= 5) for preventive and ameliorative studies respectively.

2.4. Induction of diabetes and treatments

The induction of diabetes was carried out according to the method described by Sunil *et al.* (2022). The experiment was conducted in six groups, each consisting of five animals. After overnight fasting, diabetes was induced by a single intraperitoneal injection (I.P.) of freshly prepared solution of streptozotocin (STZ; 45 mg/kg b.w.) in 0.1 M citrate buffer of pH 4.5, A glucosamine derivative of nitrosourea, which selectively destroys pancreatic islets of β-cells and results in the development of symptoms like hyperglycemia and glycosuria. Then, the diabetic rats were allowed to drink 5% (w/v) glucose solution overnight to overcome the early phase of drug-induced hypoglycaemic death. The blood glucose levels of rats were measured after 48 h, through tail tipping using a glucometer (Glucocard-01 Mini, Bengaluru). Those rats with fasting blood glucose levels >250 mg/dL were considered as diabetic and included in the study. At the time of

induction, control rats were injected with 0.2 mL of vehicle (0.1 M citrate buffer of pH 4.5) alone and STZ-treated rats were given food and water ad libitum

- Group-1: Normal control rats received an equal volume of vehicle orally (P. O.) + normal feed (unformulated feed).
- Group-2: Diabetic group received STZ 45 mg/kg/ b.w. I.P. + normal feed (unformulated feed).
- Group-3: Diabetic rats received 5% (w/w) *Cucurbita maxima* leaf formulated diet and water *ad libitum*.
- Group-4: Diabetic rats received 10% (w/w) *Cucurbita maxima* leaf formulated diet and water *ad libitum*.
- Group-5: Diabetic rats received 15% (w/w) *Cucurbita maxima* leaf formulated diet and water *ad libitum*.
- Group-6: Diabetic rats received 20% (w/w) *Cucurbita maxima* leaf formulated diet and water *ad libitum*.

During the experimental period, the blood glucose level and body weights of the rats were measured on weekly basis i.e., on days 0, 7, 14, 21, and 28. Biochemical parameters were estimated on the 28th day. The rats were sacrificed under ether anesthesia and euthanized and the blood samples were collected and preserved for further analyses.

For preventive study, diabetes was induced on the 28th day after the rats have received *Cucurbita maxima* leaf formulated diet for four weeks and the fasting blood glucose levels were estimated and consequently, the rats were sacrificed and the blood samples were collected and preserved for further analyses.

2.5. Haematological study

The whole blood of sacrificed animals were collected into EDTA bottles for analysis of haematological parameters such as hemoglobin concentration (Hb.), white blood cell count (WBC), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular heamoglobin concentration (MCHC). The determination of haematological parameters was done within two hours of sample collection using 5 Parts Hematology Analyzer – ACCULAB CBC 560+. Automated Haematological analyser by 212, Udyog Mandir No. 1 7/C, Bhagoji Keer Marg, Mahim, Mumbai – 400 016 India.

2.6. Statistical analysis

The data were analysed by the analysis of variance (ANOVA) using SPSS program (version 20 SPSS Inc., Chicago, IL, USA). The differences in parameters between the various animal groups were compared using the Bonferroni multiple comparison test (post-hoc test). The results were expressed as mean ± standard deviation (SD). P value less than 0.05 was considered as significant ($P< 0.05$). Results were presented in table, charts and graphs using MICROSOFT WORD and EXCEL.

3. Results

3.1. Preventive and ameliorative effects of C*. maxima* leaf supplemented diets on red blood cells and the differentials in stz-induced diabetic rats

The significant ($p < 0.05$) decrease in the levels of RBC, Hb and PCV observed in diabetic animals was drastically ($p < 0.05$) increased in 10%, 15% and 20% supplemented groups in the preventive treatment compared to STZ-induced diabetic control group. However, the increase was significantly ($p < 0.05$) lower compared to normal control except the level of PCV in 20%. On the other hand, the 5% supplemented group did not show significant difference at ($p < 0.05$) in RBC, Hb and PCV when compared with STZ-induced diabetic control in Table 4.9. The levels of MCH, MCV and MCHC in PV5%, PV10%, PV15% and PV20% groups rise significantly ($p < 0.05$) compared to STZ-induced diabetic control. The increase was comparable with the normal control as shown in Table 1.

Meanwhile, the RBC, Hb and PCV levels in the ameliorative supplemented groups were significantly ($p < 0.05$) increased in 5%, 10%, 15% and 20% as compared to STZ-induced diabetic control group except the PCV level in 5% supplemented that was statistically similar but numerically higher in Table 4.10. Although, the levels of MCH and MCHC in AM5%, AM10%, AM15% and AM20% compare favourably with the normal control, but MCV level did not, however, it was significantly higher at ($p < 0.05$) when compared to STZ-induced diabetic control in Table 2.

Table 1 Preventive effects of *C. maxima* leaf supplemented diets on red blood cells and the differentials in stz-induced diabetic rats

Treatment	PCV (%)	RBC (×10 cells/μL)	Hb (g/dL)	MCV (fl)	MCH (Pg)	MCHC (g/dL)
NC	34.2±2.05^{b}	6.38±0.50^{c}	11.42±0.67^{c}	81.68±15.45^{b}	28.08±0.16^{b}	34.76±1.09^{b}
PC	23.8±0.45^{c}	2.76±0.17^{a}	7.94±0.13^{a}	42.38±6.10^{a}	17.46±1.50^{a}	30.5±1.92^{a}
PV5%	26.6±0.55^{c}	3.42±0.29^{a}	9.14±0.22^{a}	65.28±6.11^{b}	24.96±1.79^{b}	33.12±0.11^{b}
PV10%	28±1.22^{a}	3.86±0.31^{b}	9.18±0.41^{a}	69.38±4.80^{b}	25.06±1.51^{b}	33.22±0.08^{b}
PV15%	29.4±1.34^{a}	3.94±0.17^{b}	9.54±0.43^{b}	74.82±6.66^{b}	27.04±3.11^{b}	33.28±0.08^{b}
PV20%	31.4±1.52^{b}	4.68±0.33^{b}	9.68±0.51^{b}	77.76±5.39^{b}	27.9±1.79^{b}	33.5±0.10^{b}

n=5; Results are in mean±standard deviation; values with different superscript down the column are significantly different at ($p < 0.05$); NC = normal control, STZC = STZ control, PV = Preventive treatment, PCV = packed cell volume, RBC = red blood cell, Hb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

Table 2 Ameliorative effects of *C. maxima* leaf supplemented diets on red blood cells and the differentials in stz-induced diabetic rats

Treatment	PCV (%)	RBC (×10 cells/μL)	Hb (g/dL)	MCV (fl)	MCH (Pg)	MCHC (g/dL)
NC	34.2±2.05^{b}	6.38±0.50^{c}	11.42±0.67^{b}	81.68±15.45^{c}	28.08±0.16^{b}	34.76±1.09^{b}
PC	23.8±0.45^{a}	2.76±0.17^{a}	7.94±0.13^{a}	42.38±6.10^{a}	17.46±1.50^{a}	30.5±1.92^{a}
AM5%	27.8±0.84^{a}	4.12±0.33^{b}	10.22±0.29^{b}	47.86±8.82^{a}	20.1±1.28^{a}	33.12±0.04^{b}
AM10%	30.8±1.64^{b}	5.42±0.33^{b}	10.26±0.54^{b}	52.72±0.75^{b}	20.02±0.35^{a}	33.16±0.05^{b}
AM15%	31.02±2.95^{b}	5.4±0.37^{b}	10.38±0.99^{b}	57.68±1.54^{b}	24.78±0.53^{b}	33.24±0.05^{b}
AM20%	31.6±2.51^{b}	5.98±0.33^{c}	10.62±0.84^{b}	69.04±1.52^{b}	25.07±0.54^{b}	33.36±0.05^{b}

n=5; Results are in mean±standard deviation; values with different superscript down the column are significantly different at ($p < 0.05$); NC = normal control, STZC = STZ control, AM = ameliorative treatment, PCV = packed cell volume, RBC = red blood cell, Hb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

3.2. Preventive and ameliorative effects of *C. maxima* leaf supplemented diets on white blood cells and the differentials in STZ-induced diabetic rats

Preventive effect *C. maxima* leaf supplemented diets on white blood cells and the differentials in STZ-induced diabetic rats are shown in Tables 3. The level of WBC was significantly ($p < 0.05$) increased after supplementation with 10%, 15% and 20% of *C. maxima* leaves in the preventive experiment as compared to the STZ-induced diabetic control but was not significant at $p < 0.05$ when compared with the normal control. However, there was only a slight increase in the 5% preventive group which was not significant at $p < 0.05$ as compared to the STZ-induced diabetic control. The rise in the lymphocytes and neutrophils levels in 5%, 10%, 15% and 20% preventive supplementations was significantly ($p < 0.05$) higher as compared with the STZ-induced diabetic control and significantly ($p < 0.05$) lower when compared with normal control. The rise in eosinophils only produced significant ($p < 0.05$) difference in 15% and 20% as compared with STZ-induced diabetic control and show no such difference in comparison with the normal control. Whereas, the rise 5% and 10% showed lower values when compared with normal control, but did not when compared to STZ-induced diabetic control.

Although, WBC levels in 10%, 15% and 20% supplementations of the ameliorative groups remained significantly ($p < 0.05$) high as compared to the STZ-induced diabetic control whereas the 5% supplantation showed no significant difference ($p > 0.05$) increase compared STZ-induced diabetic control in Table 4. The rise in the WBC of 10%, 15% and 20% in ameliorative treatment was significant at $p < 0.05$ when compared with negative control (NC). Meanwhile, the rise in lymphocytes in 10%, 15% and 20% did not produce significant difference ($p > 0.05$) when compared with the normal control except for the 5%, but the increase in 5%, 10%, 15% and 20% groups was significant at ($p < 0.05$) as compared to the STZ-induced diabetic control. Although the level of neutrophils in 5%, 10%, 15% and 20% significantly

($p < 0.05$) rise in comparison with the STZ-induced diabetic control, however, it was significantly ($p < 0.05$) lower when put side by side with the normal control. The rise in eosinophils only produced significant ($p < 0.05$) difference in 15% and 20% as compared with STZ-induced diabetic control and show no such difference in comparison with the normal control. Whereas, the rise 5% and 10% showed lower values when compared with normal control, but did not when compared to STZ-induced diabetic control.

Table 3 Preventive effects of *C. maxima* leaf supplemented diets on white blood cells and the differentials in STZ-induced diabetic rats

Treatment	WBC (10^3/L)	Lymphocytes (%)	Neutrophil (%)	Eosinophil (%)
NC	7.32±1.53^b	62±2.45^c	56.4±5.68^c	4.2±1.48^b
PC	3.58±0.80^a	41±4.12^a	33.2±2.05^a	0.44±0.42^a
PV5%	4.3±0.90^a	52.4±1.34^b	41.6±1.52^b	0.74±0.42^a
PV10%	6.06±0.65^b	54.8±2.05^b	41.9±1.52^b	1.88±0.76^a
PV15%	6.28±0.45^b	55.2±2.17^b	43.2±0.84^b	2.47±1.05^b
PV20%	6.7±0.19^b	55.5±1.22^b	44.6±1.52^b	2.6±1.52^b

n=5; Results are in mean±standard deviation; values with different superscript down the column are significantly different at ($p < 0.05$); NC = normal control, STZC = STZ control, PV = Preventive treatment, WBC = white blood cell.

Table 4 Ameliorative effects of *C. maxima* leaf supplemented diets on white blood cells and the differentials in STZ-induced diabetic rats

Treatment	WBC (10^3/L)	Lymphocytes (%)	Neutrophil (%)	Eosinophil (%)
NC	7.32±1.53^c	64±2.45^c	56.4±5.68^c	4.2±1.48^b
PC	3.58±0.80^a	41±4.12^a	33.2±2.05^a	0.44±0.42^a
AM5%	4.04±0.53^a	53.4±1.52^b	35±3.67^a	1.83±0.88^a
AM10%	4.36±0.49^b	60.6±2.07^c	37.2±3.83^a	1.6±1.34^a
AM15%	4.92±0.20^b	61±2.45^c	43.4±0.55^b	2.64±0.54^b
AM20%	5.2±0.30^b	62.2±0.84^c	45.8±0.84^b	2.9±0.96^b

n=5; Results are in mean±standard deviation; values with different superscript down the column are significantly different at ($p < 0.05$); NC = normal control, STZC = STZ control, AM = ameliorative treatment, WBC = white blood cell.

4. Discussion

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including STZ on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, normal functioning and histomorphology of the organs (Magalhaes *et al.,* 2008). The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins (Oyedemi *et al.,* 2011). Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (Arun and Ramesh, 2002). In this study, the red blood cells parameters such as Hb, MCHC, MCH, and MCV were studied to investigate the beneficial effect of *C. maxima* leaves fortified diet on the anaemic status of the diabetic rats. The levels of RBC, Hb, and MCHC in the diabetic animals were drastically reduced which may be attributed to the obstructions to the normal body systems. This observation agrees with report of Baskar *et al. (2006)* who reported antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats. The alterations of these parameters are well known to cause anaemic condition in man (Balasubraimanian *et al.,* 2009). Following *C. maxima* leaves fortified diet administartion, the level of RBC and its related indices were appreciably improved in preventive and ameliorative treatments. This gives an indication that the plant extract may contain some active compounds that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a

glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006). The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC (Abu-Zaiton, 2010). These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. Though, the action mechanism of this plant is not investigated in this study. However, it may be attributed to the ability of plant extract to lower lipid peroxidation level brought about by STZ that causes haemolysis of erythrocytes (Ashafa *et al.,* 2009). Previously, this plant revealed the presence of flavonoids, proanthocyanidins, tannins, phenols and flavonols that have been reported to possess strong antioxidant capacity (Akah *et al.,* 2007), therefore, could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals reported by Faure *et al*. (1991).

Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body. The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count and its differentials such as monocytes, lymphocytes and neutrophils. The reduction of these parameters in the diabetic rats could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection (Oyedemi *et al.,* 2010). Consequentially, they might have effects on the immune system and phagocytic activity of the animals (Torell *et al*., 1986). The white blood counts and its related indices were significantly restored to near normal after *C. maxima* leaves fortified diet at both preventive and ameliorative treatments. The presence of some phytochemicals with ability to stimulate the production of white blood count in the extract could be responsible for the observed result in the treated rats (Akinpelu *et al,* 2008). The extract at both treatments significantly improved the levels of WBC, lymphocytes, eosinophils and neutrophils as compared with non-treated group indicating the haematinic effect of the *C. maxima* leaves.

5. Conclusion

The results obtained through these investigations pointed to both preventive and ameliorative haematinic and erythropoietic potentials of *Cucurbita maxima* leaf in a diabetic state.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All ethical proceedings regard animal studies set by the department of Biochemistry Federal University Wukari were strictly followed throughout this experiment.

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