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Effects of some green leafy vegetables on the haematological parameters of diabetic rats

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ABSTRACT

The effect of some green leafy vegetable diets on haematological parameters of streptozotocin-induced diabetic rats was investigated. 16% vegetable inclusion (Occimum gratissimum,Pterocarpus soyauxii,Corchorus olitorius and Vernonia amygdalina) in the basal diets of streptocotozin-induced diabetic rats for 21 days resulted in modulation of haematological parameters. The results showed that there was a significant decrease (p<0.05) in the levels of Packed cell volume(PCV), Haemoglobin (Hb),white blood cell(WBC), red blood cell(RBC), platelets and neutrophils counts of the diabetic rats without vegetable-formulated diets when compared to the control. However, a significant increase of these hematological indices was observed in the groups fed with vegetable-diet. Lymphocytes, eosinophils, monocytes and basophils also showed significant difference(p<0.05) with increase in lymphocyte count. Among these vegetables, Pterocarpus soyauxii seems to be most promising. These results therefore suggest that green leafy vegetables are capable of normalising the hematological abnormalities associated with pathophysiology of diabetes mellitus.

Keywords: Leafy vegetable, Hematology, Diets, Diabetic rats.

INTRODUCTION

Haematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health [1]. It is an established fact that chronic diseases affect the blood cells adversely. Analysis of blood parameters is relevant to risk evaluation of alterations of the haematological system in humans [2]. Damage mediated by free radicals results in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions, which have generally been considered to be linked with many chronic health problems such as diabetes, cancers, inflammation, aging and atherosclerosis. Although almost all organisms possess antioxidant defense and repair systems that have evolved to protect them against free radicals, these systems are insufficient to protect them completely against oxidative damage [3].

Green leafy vegetables such as *Ocimum gratissimum*, *Pterocarpus soyauxii*, *Corchorus olitorius* and *Vernonia amygdalina* contain compounds that are valuable antioxidants and protectants. The main protective action of vegetables has been attributed to the presence of antioxidants, especially antioxidant vitamins including ascorbic acid, α-tocopherol, β-carotene and phenolics [4;5].

Several green leafy vegetables abound in tropical Africa that are utilized either as condiments or spices in human diets. These vegetables could be harvested at all stages in the process of growth, and could be harnessed as a cheaper and safer means of managing haematological abnormalities associated with the pathophysiology of diabetes mellitus. As a result of inadequate information on the comparative studies of these green leafy vegetables in relation to their contribution in the hematology of diabetic subjects, this study sought to investigate their effects on some haematological parameters of diabetic rats.

MATERIALS AND METHODS

Experimental animals and design

Wistar strain albino rats weighing between 180-200g were obtained from the Animal Unit of the Department of Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Oyo State of Nigeria. They were acclimatized for two weeks, during which period they were maintained *ad libitum* on commercial diet (Top feed, Nigeria). The rats were administered Streptozocin dissolved in normal saline (65mg/kg b.w) intraperitonially. Subsequently, rats with blood glucose level greater or equal to 230mg/dl were divided into 6 groups. The diabetic rats were subsequently divided into six treatment groups. Animals in groups 1 and 2 were fed the basal diet (Skimmed milk (42%), corn starch, Premix, and soy oil. While groups 3 to 6 were fed with basal diet with 16% inclusion of vegetables (at the expense of corn flour).Group 1 serves as the normal control while other groups were diabetic. The feeding was done ad libitum. All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Tropical vegetables

Pterocarpus soyauxiii, Ocimum gratissimum, Corchorus olitorius and Vernonia amygdalina were obtained locally from open forest at Akungba Akoko, Ondo State, Nigeria and identified by the Department of Crop Science and Protection, Federal University of Technology, Akure, Ondo State,Nigeria.

The leaves were separated from the inedible portions. These were subsequently chopped into small pieces, washed in water and then sun-dried. The dried vegetables were subsequently blended with Kenwood blender into fine powder. Grinded forms were subsequently used for the diet formulation.

Grouping of Animals

The study of the effects of the medicinal plants on the oxidative status of STZ-induced diabetic rats was done by putting five rats per group.
Group 1: Normal Control
Group 2: Diabetic control
Group 3: *Ocimum gratissimum* fed diabetic rats
Group 4: *Pterocarpus soyauxiii* fed diabetic rats
Group 5: *Corchorus olitorius* fed diabetic rats
Group 6: *Vernonia amygdalina* fed diabetic rats

Blood collection

At the end of the monitoring phase, the rats were sacrificed by cervical dislocation and the blood was obtained through heart puncture for haematological analysis.

Haematological estimations

The blood samples were obtained via cardiac puncture, collected into heparinized tubes and were immediately used for determination of haematological parameters. Total red blood cell and white blood cell counts were estimated according to the visual method of [6]. The percentage packed cell volume was determined according to the hematocrit method of [7] while the blood haemoglobin concentration in all samples was estimated according to the cyanomethaemoglobin method of [7].

Differential White Blood Cell counts

These were estimated using the method of [8]. A dry micropipette was used to suck in blood from the blood sample bottle, a small drop of blood was applied to one end of a slide and quickly placed on the bench holding it in position, the end of the second slide was then placed in the drop and held there until the blood had spread across it. It was then drawn slowly over the whole length of the first slide being held at an angle of 45°. After the blood had spread, it was dried before staining with Leishman's stain. The film which was washed off in a gentle stream of water was dried with filter paper and examined under low and high power microscope and the different kinds of cells counted.

Statistical analysis

The data are expressed as mean± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA). Difference were considered to be statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Table 1: Effects of vegetable included diets on the PCV, Hemoglobin, WBC, RBC, and platelet in all groups of rats

Group	PCV(%)	Hb(g/dl)	WBC(10*3/µl)	RBC(10*6/µl)	Platelet(10*9/L)
Normal Control	42.00±0.58^{c}	137.33±0.33^{c}	13.40±0.06^{c}	7.79±0.12^{c}	596.67±4.41^{d}
Diabetic Control	24.00±1.73^{a}	86.00±8.08^{a}	6.25±0.49^{b}	4.41±0.34^{a}	297.00±37.53^{c}
O.G fed rats+ diabetic	31.33±0.88^{b}	111.00±5.19^{b}	3.60±0.35^{a}	6.08±0.06^{b}	168.00±3.46^{b}
P.S fed rats+diabetic	50.00±1.73^{d}	179.00±9.82^{d}	4.95±1.13^{ab}	9.31±0.37^{d}	824.00±65.82^{e}
C.O fed rats+diabetic	34.67±0.33^{b}	131.33±0.33^{c}	15.55±0.32^{d}	7.49±0.40^{c}	297.67±2.60^{c}
V.A fed rats+diabetic	24.00±1.16^{a}	85.67±3.76^{a}	4.65±0.14^{ab}	4.48±0.27^{a}	40.00±1.73^{a}

The result was presented as Mean±SEM .Values with different superscript are significantly different at p<0.05.

Table 2: Effects of vegetable diet inclusion on white blood cell's differentials in all groups of rats

Group	Neutrophils(%)	Lymphocytes(%)	Eosinophils(%)	Monocytes(%)	Basophils(%)
Control	50.00±0.58^{e}	41.00±0.58^{a}	8.16±1.16^{b}	2.50±0.29^{b}	2.00±0.00^{b}
Diabetic control	40.00±0.58^{c}	44.00±1.00^{ab}	11.67±0.88^{c}	2.00±0.58^{ab}	1.67±0.33^{b}
O.G fed rats	24.67±0.33^{a}	72.33±0.67^{e}	1.00±0.00^{a}	1.67±0.33^{ab}	0.33±0.03^{a}
P.S fed rats	47.00±0.58^{d}	49.33±1.20^{bc}	1.33±0.33^{a}	1.00±0.00^{a}	0.00±0.00^{a}
C.O fed rats	50.67±1.45^{e}	50.67±3.48^{c}	1.00±0.00^{a}	1.33±0.33^{ab}	0.33±0.33^{a}
V.A fed rats	30.00±1.16^{b}	65.00±2.89^{d}	1.33±0.33^{a}	1.33±0.33^{ab}	0.72±0.21^{a}

The result was presented as Mean±SEM .Values with different superscript are significantly different at p<0.05.

Table 1 shows the effects of vegetable-included diets on the PCV, Hemoglobin, WBC, RBC, and platelet in all groups of rats.

Diabetic rats fed with basal diet showed statistically significant decrease (P<0.05) in levels of PCV, Hb, WBC, RBC and platelet when compared to normal rats on basal diet. On the other hand, inclusion of leafy vegetables restored most of the derangement in heamatological parameters of diabetic rats. Diabetic rats placed on *Occimum gratissimum-, Pterocarpus soyauxii-* and *Corchorus soyauxi*-included diets showed significantly increased (P<0.05) PCV, Hb and RBC levels when compared with diabetic rats on basal diets. Rats on *Pterocarpus soyauxii*-included diets also showed significantly increased platelets level compared with the normal control and diabetic groups on basal diets. *Pterocarpus soyauxii* fed-rats notably had the highest levels of PCV, Hb, RBC and platelets when compared to other groups.

Table 2 shows the effects of the vegetable diet inclusion on white blood cells differentials in all groups. Blood Eosinophils were significantly increased (P<0.05) in diabetic-untreated group compared with normal rats. This alteration was reversed in diabetic rats placed on *Occimum gratissimum*-included diet. Also, statistically significant reduction (P<0.05) in Basophils levels was observed in diabetic rats fed with diet containing all vegetables.

The blood is a vital fluid, which contains the Red Blood Cells (RBC's), White Blood Cells (WBC's) and platelets suspended in the serum in homeostatic concentrations. The circulatory blood volume makes up about 8% of the weight of an average man. The blood cells take up about 45% of the blood, while plasma constitutes about 55 % [9]. The blood is important for pulmonary and tissue respiration, as a medium of endocrine and neurohumoral transmissions, biotransformation and metabolic excretion, [10] nutritional and immunological processes, as well as homeostatic responses [11]. Reactive oxygen species have been implicated in the mechanism of damage of red blood cells in diabetic patients [12,13,14]. As a result, haematological complications develop which consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets [15].

Packed Cell Volume measures the percentage by volume of packed RBC's in a whole blood sample after centrifugation. The significant decrease in the level of packed cell volume (PCV) in diabetic control rats **(Table 1)** may be as a result of the cellular damage on the erythrocyte membrane as a result of oxidative stress by streptozotocin (STZ) [16,17]. Diabetic rats fed with basal diet also showed significant decrease (p<0.05) in values of Hb, WBC, RBC and platelet when compared to normal rats on basal diet. Haemoglobin test measures the amount of HB in grams in 1 dl of whole blood and provides an estimate of oxygen carrying capacity of the RBC's. Red blood cell counts can be a factor in erythropoietin process. On the other hand, white blood cell count can indicate that there is a disease or condition affecting white blood cells, but it cannot determine the underlying cause. It is also related to the immune system and bone marrow. Platelet count is used to diagnose and/or monitor bleeding and clotting disorders. Since, a marked reduction was observed in these aforementioned parameters this may be an indication of anaemia in diabetic rats. It has been suggested that anaemia occurrence in diabetes mellitus is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia [18,19]. Oxidation of these glycosylated membrane proteins and hyperglycaemia in diabetes mellitus cause an increase in the production of lipid peroxides causing a haemolysis of RBC through many pathological consequences [20]. The major pathological consequences of free radical induced membrane lipid peroxidation include increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity [20]. On other hand, inclusion of leafy vegetables significantly increased ($p<0.05$) these parameters to their normal levels and even higher in some when compared to diabetic rats. This is in line with the findings of Eteng et al. [21] who reported the reversal of anemia in cadmium toxicity after the supplementation of diets. This may be important in promoting health because of their rich sources of nutrients as reported by Gupta and Prakash [22]. White blood cell differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes. Neutrophils attack and destroy bacteria in the blood [23]. As shown in Table 2, the significant increase in neutrophils and eosinophils levels of diabetic rats may be attributed to diabetic complications. It has been demonstrated that severity of diabetes and the development of retinopathy are associated with increased numbers of polymorphonuclear leukocytes [24]. The reversal of this derangement in rats placed on vegetable-included diets may signify the protective effect of the intervention. Lymphocytes are the main effector cells of the immune system. The increase in the lymphocyte count in rat place on vegetable diet may be an indication of immunostimulation.

CONCLUSION

In conclusion, the increase in red blood cell, packed cell volume (PCV or haematocrit), total white blood cell, platelet and lymphocyte counts following administration of the supplemented vegetable diets may signify the positive effects of vegetables on the haemopoietic system of experimental rats and might be capable of improving the hematological abnormalities associated with pathophysiology of diabetes mellitus.

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