



## AMELIORATIVE EFFECT OF L-ASCORBIC ACID AND $\alpha$ -TOCOPHEROL CO-ADMINISTRATION ON BLOOD GLUCOSE LEVELS AND SOME HAEMATOLOGICAL INDICES ON HIGH FED FAT DIET-INDUCED DIABETES ON WISTAR RATS

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### ABSTRACT

This study investigated the effects of L-ascorbic acid and  $\alpha$ -tocopherol co-administered on blood glucose levels and haematological indices on high fed fat diet induced diabetes on Wistar rats. Diabetes was induced by the administration of high fed fat diet 10 % groundnut oil, 20 % groundnut mill and 2 % cholesterol for eight weeks. Thereafter the animals were randomly assigned into 5 groups of 5 rats each. Group 1, negative control, was administered 1ml distilled water. Group 2 was administered 100 mg/kg L-ascorbic acid. Group 3 administered 10 mg/kg  $\alpha$ -tocopherol. Group 4 co-administered 100 mg/kg L-ascorbic acid and 10 mg/kg  $\alpha$ -tocopherol. Group 5 administered glibenclimide 1 mg/kg, served as positive control. All treatments were administered orally for a period of four weeks. The blood glucose levels, significantly decreased ( $P < 0.05$ ) in the groups administered L-ascorbic,  $\alpha$ -tocopherol and co-administration when compared with the control. Also in relation to the erythrocyte indices, there was no significant change in the two tested vitamins when compared to the control. However, there was a significant increase ( $p < 0.05$ ) in the white blood cells count of rats treated with  $\alpha$ -tocopherol after 4 week of treatment when compared with the control. However, there was a significant increase ( $p < 0.05$ ) and decrease in the platelet count when compared with the control. L-ascorbic acid and 10 mg/kg  $\alpha$ -tocopherol 100 mg/kg were found to have potent anti-diabetic effects.

**Keywords:** L-ascorbic,  $\alpha$ -tocopherol, haematological indices, blood glucose level

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### INTRODUCTION

Diabetes is a common metabolic disorder characterized by hyperglycemia due to an absolute or relative insulin deficiency [1]. It affects essential biochemical pathways of the body including carbohydrate, protein, and lipid metabolisms. The World Health Organization (WHO), estimated that there were 171 million people in the world with diabetes in the year 2008 and this is projected to increase by over a 100% to 366 million by 2030 [2]. Diabetes is associated with reduced life expectancy, significant high mortality and diminished quality of life. In 2005 an estimated 1.1 million people died from diabetes and diabetes complications [3]. Its prevalence is rising globally, including the rural Nigerian population [4].

Epidemiological reports has highlighted on the fact that low- and middle-income countries will bear the brunt of the increase and that Africa will contribute significantly to this rise. In Africa, 40% of people with diabetes live in low and middle income countries causing 5% of the deaths globally each year. This is likely to increase by more than 50% in the next 10 years if urgent action is not taken [5]. The challenges and thus, the solutions in the provision of healthcare that would improve outcome for diabetes in low and middle income countries are many and can be found at multiple levels. Patient-related factors are of extreme importance and these range from low levels of self-management practices, lack of adherence to lifestyle changes to medication and lack of faith in the conventional management procedures. Many African populations still regard alternative healing systems

as the primary source of healthcare or alternatively, consult both traditional or folk healers that usually promote the concept that diabetes is curable and who are also reluctant to refer clients to medical practitioners [6, 7]. This, however, is undermined by two key factors; the high cost of drugs and recommended foods as well as the psychosocial burden imposed by the daily oral hypoglycaemic drugs therapeutic routines. Moreover, spiritual causal theories of diabetes such as sorcery and witchcraft are still found in many African populations, particularly in rural communities [8].

However, it appears that oxidative stress and lipid peroxidation are central factors and major drivers in the etiology of diabetes and its complications [9] and that reactive oxygen species (ROS) and lipid peroxidation products, operates through multiple pathways to cause metabolic perturbations of essential biochemical pathways of the body [9]. In diabetic condition, elevated levels of blood glucose (hyperglycemia) and insulin (hyperinsulinemia) may provide a pro-oxidant environment [10]. Individuals with diabetes do not have sufficient antioxidant defenses [11, 12] as hyperglycaemia has been reported to impair their antioxidant defenses [13]. Under hyperglycaemic conditions, superoxide dismutase is glycated resulting in a decrease in its activity. In addition, the activity of the glutathione redox cycle (GR cycle) is decreased due to the impaired activation of the pentose phosphate pathway [14]. Accordingly, it will be expected that exogenous antioxidant supplementation is desirable in patients with diabetes mellitus but surprisingly available

exogenous antioxidants do not seem to confer any decisive benefit [15, 16, 17].

The excessive consumption of high cholesterol diet has been associated with an increased incidence of obesity [18]. This is because obesity induces pathologies with high mortality, such as complications of dyslipidaemia, diabetes mellitus, arthritis, hypertension, myocardial infarction, stroke, non-alcoholic fatty liver disease, cirrhosis, steatohepatitis and hepatocellular carcinoma [19-23]. Moreover with the alarming increase in the prevalence of obesity worldwide, obesity has become a major health-care burden, not just in terms of the increased risk for the aforementioned associated diseases, but also in the economic costs to health-care providers. It is generally accepted that the tremendous rise in the prevalence of obesity across the globe is driven primarily by a combination of increased calorie intake and decreased physical activity [24] and that it is strongly influenced by genetic background [25]. Although the associated diseases or the obesity-induced pathologies are primarily driven by metabolic dysfunctions, characterized by abnormalities in glucose metabolism and thyroid function, they are enhanced by formation of oxidative stress, lipid peroxidation and hypercholesterolaemia [26-29].

This study reports on the ameliorative effects of L-ascorbic acid and  $\alpha$ -tocopherol co-administration on blood glucose levels and haematological parameters on high fat diet-induced diabetes in Wistar rats.

## MATERIALS AND METHODS

### Chemicals

All chemicals were obtained commercially and were of analytical grade: Cholesterol (Mumbai, India, M.W. 386.67, CAS No. 57-88-5, LoT No. 100413). L-ascorbic acid and  $\alpha$ -tocopherol from Zayo Stigma Aldrich Company, Jos, Nigeria.

### Animals

Twenty five (25) Wistar rats of either sex (weighed between 200- 250 g) were obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria. The rats were maintained on standard laboratory animal feed (Vital Feeds Company, Kaduna, Nigeria) and water *ad libitum*, and housed in polypropylene cages at room temperature throughout the study. The Principle of laboratory animal care (NIH publication No 85-23) guideline and procedures were followed in this study (NIH publication reserved, 1985).

### Induction of obesity and diabetes mellitus

The animals were fasted for 16-18 hr before the commencement of the experiment, but were allowed water *ad libitum*. The normal groups were fed with standard animal feeds only, while the high fat-diet groups were fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol/kg/day) for the induction of obesity, diabetes and oxidative stress for the experimental period, which lasted for eight weeks. Rats

with blood glucose levels greater than 120 mg/dL were considered diabetic and used for the study.

### Experimental Design

In the experiment, a total of 25 rats were used. The rats were randomly divided into 5 groups of 5 rats each as follows:

**Group 1:** Fed with standard animal feeds and high fat diet (10 % groundnut oil, 20 % groundnut mill and 2 % cholesterol) administered distilled water as control orally for four weeks.

**Group 2:** Fed with standard animal feeds and high fat diet (10 % groundnut oil, 20 % groundnut mill and 2 % cholesterol) administered 100 mg/kg L-ascorbic acid orally for four weeks administered once daily.

**Group 3:** Fed with standard animal feeds and high fat diet (10 % groundnut oil, 20 % groundnut mill and 2 % cholesterol) administered 10 mg/kg  $\alpha$ -tocopherol orally for four weeks administered once daily.

**Group 4:** Fed with standard animal feeds and high fat diet (10 % groundnut oil, 20 % groundnut mill and 2 % cholesterol) administered 100mg/kg L-ascorbic acid and 10mg/kg  $\alpha$ -tocopherol orally for four weeks administered once daily.

**Group 5:** fed with standard animal feeds and high fat diet (10 % groundnut oil, 20 % groundnut mill and 2 % cholesterol) administered glibenclamide 1mg/kg orally for four weeks administered once daily.

### Determination of blood of glucose level and haematological indices

Fasting blood glucose level was determined at interval of 1, 2, 3 and 4 weeks, using the glucose oxidase principle [30] with a digital glucometer (Accu-check Advantage, Roche Diagnostic, Germany), by bleeding the rats tails and placing a drop of blood on the glucose strip fixed in the glucometer, the results were expressed in mg/dl [31]. After the last day of administration the animals were euthanized and blood samples were drawn from the heart of each by cardiac puncture into plain tubes with drops of Ethylene diamine tetraacetic acid (EDTA) for determination of blood parameters: red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), platelets count, white blood cell count (WBC) and its differential counts using the method of [32].

### Statistical analysis

Data obtained from each group were expressed as mean  $\pm$  SEM. The data were statistically analyzed using (ANOVA) with Tukey's *post-hoc test* to compare the levels of significant between the control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software and Microsoft Excel (2007). The values of  $P \leq 0.05$  were considered as significant.

**RESULTS**

Table 1 showed the results of the effects of L-ascorbic acid and  $\alpha$ -tocopherol (100 mg/kg and 10 mg/kg) and co-administered respectively with high fat diet-fed rats groups and untreated group. The high fat diet-fed rats showed

significant ( $P < 0.05$ ) increase in the blood glucose levels after eight weeks of administration, when compared to the untreated group. At 1, 2, 3 and 4 weeks of administration of L-ascorbic acid and  $\alpha$ -tocopherol showed significant ( $P < 0.05$ ) decreased in blood glucose level compared to the control at the tested doses.

**Table 1:** Effects of L-ascorbic acid and  $\alpha$ -tocopherol co-administered on rat fed with high-fat diet on blood glucose levels after 4 weeks of administration.

Group(N=5)	Treatment	Initial	Week 1	Week 2	Week 3	Week 4
1	Control	142.2 ± 0.32	139.3 ± 3.11	130.6 ± 1.28	144.6 ± 2.21	136.4 ± 2.09
2	Ascorbic acid (100 mg/kg)	140.0 ± 0.11	108 ± 1.01 <sup>ns</sup>	99.1 ± 1.02 <sup>a</sup>	78.8 ± 2.01 <sup>a</sup>	75.8 ± 2.21 <sup>a</sup>
3	$\alpha$ -Tocopherol (10 mg/kg)	139.2 ± 0.21	102 ± 1.01 <sup>ns</sup>	76.3 ± 2.13 <sup>a</sup>	72.8 ± 1.93 <sup>a</sup>	73.6 ± 1.88 <sup>a</sup>
4	Ascorbic acid(100mg/kg) and $\alpha$ -Tocopherol (10 mg/kg)	141.4 ± 0.17	56 ± 1.23 <sup>a</sup>	70.8 ± 1.03 <sup>a</sup>	60.5 ± 1.88 <sup>a</sup>	63.4 ± 2.22 <sup>a</sup>
5	Glibenclimide (1mg/kg)	142.0 ± 0.18	58.2 ± 0.06 <sup>a</sup>	68.2 ± 2.02 <sup>a</sup>	59.6 ± 1.52 <sup>a</sup>	60.1 ± 2.07 <sup>a</sup>

Table 1 showed the results of the effects of L-ascorbic acid and  $\alpha$ -tocopherol (100 mg/kg and 10 mg/kg) and co-administered respectively with high fat diet-fed rats groups and untreated group on blood glucose levels. The high fat diet-fed rats showed significant ( $P < 0.05$ ) increase in the blood glucose levels after eight weeks of administration, when compared to untreated group. At 1, 2, 3 and 4 weeks of administration of L- ascorbic acid and  $\alpha$ - tocopherol showed significant ( $P < 0.05$ ) decreased in blood glucose level compared control at the tested doses.

**Table 2:** Effects of L-ascorbic acid and  $\alpha$ -tocopherol co-administered on rat fed with high-fat diet on erythrocytes indices of diabetic Wistar rats 4 weeks of administration.

Group(n=5)	Treatment	RBC × 10 <sup>12</sup> / L	Hb (gm/dl)	PCV (%)	PLT × 10 <sup>12</sup> / L
1	Control	6.86±0.21	12.0±0.54	35.8±1.56	8.78±0.24 <sup>ns</sup>
2	Ascorbic acid (100 mg/kg)	6.88±0.30 <sup>ns</sup>	11.1± 1.29 <sup>ns</sup>	32.2±4.19 <sup>ns</sup>	7.78±0.24 <sup>a</sup>
3	$\alpha$ -tocopherol (10 mg/kg)	7.10±0.19 <sup>ns</sup>	10.4±0.86 <sup>ns</sup>	31.2±2.75 <sup>ns</sup>	8.08±0.24 <sup>ns</sup>
4	Ascorbic acid(100mg/kg) and $\alpha$ -tocopherol (10 mg/kg)	7.04±0.18 <sup>ns</sup>	11.1±0.98 <sup>ns</sup>	32.8±2.92 <sup>ns</sup>	7.00±0.07 <sup>a</sup>
5	Glibenclimide (1 mg/kg)	6.75±0.25 <sup>ns</sup>	14.5±0.95 <sup>ns</sup>	32.8±2.92 <sup>ns</sup>	6.80±0.30 <sup>a</sup>

Table 2 above showed the results of the effects of L-ascorbic acid and  $\alpha$ -tocopherol (100 mg/kg and 10 mg/kg) and co-administered respectively to high fat diet-fed rats groups and untreated group on erythrocytes indices. However, there was a significant decrease ( $P < 0.05$ ) in the platelet count when compared with the control.

**Table 3:** Effects of L-acid and  $\alpha$ -tocopherol co-administered on rat fed with high-fat diet on leucocytes counts of diabetic Wistar rats 4 weeks of administration

Group (n=5)	Treatment	WBC $\times 10^9$ /L	Neutrophils (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)
1	Control	7.96 $\pm$ 0.22	15.6 $\pm$ 1.38	2.00 $\pm$ 0.32	80.0 $\pm$ 1.58	2.40 $\pm$ 0.51
2	Ascorbic acid (100 mg/kg)	4.68 $\pm$ 0.22 <sup>a</sup>	17.5 $\pm$ 1.04 <sup>ns</sup>	2.00 $\pm$ 1.15 <sup>a</sup>	78.2 $\pm$ 1.55 <sup>ns</sup>	0.60 $\pm$ 0.24 <sup>ns</sup>
3	$\alpha$ -Tocopherol (10 mg/kg)	5.38 $\pm$ 0.23 <sup>a</sup>	14.2 $\pm$ 1.02 <sup>ns</sup>	1.60 $\pm$ 0.24 <sup>a</sup>	81.8 $\pm$ 1.11 <sup>ns</sup>	2.40 $\pm$ 0.24 <sup>ns</sup>
4	Ascorbic acid (100 mg/kg) and $\alpha$ -tocopherol (10 mg/kg)	5.67 $\pm$ 0.12 <sup>a</sup>	17.8 $\pm$ 0.97 <sup>ns</sup>	1.40 $\pm$ 0.40 <sup>a</sup>	79.0 $\pm$ 1.00 <sup>ns</sup>	1.80 $\pm$ 0.37 <sup>ns</sup>
5	Glibenclimide (1mg/kg)	5.10 $\pm$ 0.20 <sup>ns</sup>	13.5 $\pm$ 1.50 <sup>ns</sup>	2.00 $\pm$ 0.00	81.5 $\pm$ 0.50 <sup>ns</sup>	3.00 $\pm$ 1.00 <sup>ns</sup>

Table 3 above showed the results of the effects of L-ascorbic acid and  $\alpha$ -tocopherol (100 mg/kg and 10 mg/kg) and co-administered respectively to high fat diet-fed rats groups and untreated group on leucocyte counts. In relation to the eosinophil count there was a significant decrease when compared with the control, while there is no significant change in the neutrophil, lymphocyte and monocytes counts when compared with the control untreated group.

## DISCUSSION

Results obtained from the present study indicated that, there was a significant decrease in the blood glucose levels following administration of L-ascorbic and  $\alpha$ -tocopherol when compared with the control.

However, treatment of diabetic animals with 100 mg/kg of L-ascorbic acid and 100 mg/kg of  $\alpha$ -tocopherol significantly decreased the blood glucose concentration, with better effect recorded after week 2 and 3, and week 4 respectively when compared with corresponding diabetic untreated animals.

The possible mechanism involved in the hypoglycaemic action of L-ascorbic acid and  $\alpha$ -tocopherol may be stimulation of insulin secretion by the pancreas or/and enhance insulin sensitivity in various organs especially the muscles by promoting glucose uptake and metabolism inhibiting hepatic gluconeogenesis. As regards to glibenclamide, it has been reported to stimulate insulin secretion from pancreatic  $\beta$ -cells and also reduce hepatic glucose production resulting in reduced blood glucose level [33]. Furthermore, the improvement observed with glibenclamide administration in diabetic animals may be evident by significant decrease in the blood glucose levels; previous studies [34, 35] have demonstrated that glibenclamide is able to maintain prolonged increase in serum insulin as it binds to receptors on the surface of pancreatic  $\beta$ -cells resulting in the cell membrane creating an influx of calcium ions and subsequent release of insulin [36].

Comparable effect of L-ascorbic acid,  $\alpha$ -tocopherol and glibenclamide in the study may suggest similar mechanism of action. Oxidative stress induced by reactive oxygen species (ROS), which are generated due to hyperglycaemia has been implicated in the onset and

progression of diabetes mellitus and its related complications [37, 38, 39]. Hyperglycemia in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increases the levels of free radicals [40, 41]. L-ascorbic acid and  $\alpha$ -tocopherol are potent antioxidants, shown to have good free radical scavenging capacity because of their unique structure. Therefore, hypoglycaemic effect of L-ascorbic acid and  $\alpha$ -tocopherol may also be attributed to their strong antioxidant property [42, 43]. Also L-ascorbic acid and  $\alpha$ -tocopherol may have the ability to quench the superoxide and other free radical anions, which are released in diabetes due to abnormal glucose metabolism, hence resulting in a decrease in blood glucose concentration in diabetic animals as was observed in the present study. The decrease in total white blood cell count may be indicative of an anti-infective effect while the increase in platelet and white blood cell counts with increasing blood glucose in patients with type 1 diabetes mellitus could be as a result of a stress response. However, there was a decrease observed in the white blood cell count in the groups treated with L-ascorbic acid and  $\alpha$ -tocopherol when compared with the control. As regards to the platelet count, there was a significant decrease when compared with the control. The WBC counts have been reported to correlate positively with platelet counts, which may suggest that a shared mechanism drives both the elevated platelet and WBC counts in patients with this syndrome [44]. Clinically, elevated platelet counts are frequently seen in diabetics with a long duration of disease. Previous report seems to suggest the possibility that elevated platelet count could be used as a prognostic indicator of future diabetic complications [45].

In conclusion, L-ascorbic acid and  $\alpha$ -tocopherol administered single or in combination have effects on blood glucose level, platelet count and white blood cells on high fat diet-induced type 2 diabetes.

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