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Antioxidant enzymes and minerals status of diabetic subjects supplemented with antioxidant rich supplement (*Alphabetic*)

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ABSTRACT

Objective: Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action. Increasing evidence suggests that oxidative stress plays a role in the pathogenesis of DM and its complications. The present study examines the effects of antioxidant rich supplement (Alphabetic) on antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)] and minerals [Selenium (Se), Copper (Cu), Zinc (Zn), Manganese (Mn), and Chromium (Cr)] status of one hundred (100) diabetic subjects attending Usmanu Danfodiyo University Teaching Hospital for at least one year and the results were compared with those of fifty (50) non-supplemented diabetic subjects and fifty (50) apparently healthy non-diabetic subjects.

Methods: CAT activity was assessed using chemical reactivity method. SOD and GPx activities were assessed using Cayman's Assay Kits according to manufacturer's instructions. Antioxidant Minerals Levels were estimated by atomic absorption spectroscopy.

Results: The results revealed that the supplementation with Alphabetic significantly ($P < 0.05$) increased SOD, CAT, GPx activities and also increased the blood levels of Se, Zn, Mn, and Cr compared with the non-supplement group.

Conclusion: These findings suggested that supplementation with antioxidant rich supplements might reduce the risk of oxidative stress associated with DM complications.

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Alphabetic; Antioxidant; Diabetes; Enzymes; Minerals

Introduction

Diabetes mellitus (DM) is the most rapidly growing chronic disease in the world. DM is a group of metabolic disorders characterized by persistent hyperglycemia and disturbances in the metabolism of fuel molecules as a result of absolute or relative deficiency in insulin secretion or/and insulin action [1].

The prevalence of DM has risen dramatically over the last three decades and the number people with DM aged 20–79 years was estimated to rise to about 642 million worldwide by 2040 [2]. The estimated annual global health expenditure attributable to diabetes ranged from USD 612 billion to USD 1,099 billion [3]. In Nigeria, about 1.7 million people are living with DM [4]. The adoption of a sedentary lifestyle, the consumption of non-traditional foods, and a genetic predisposition to the disease are thought to be the major risk factors of the epidemic [5,6]. Presently, DM is managed by diet, exercise, and oral hypoglycemic drugs [7].

Diabetic patients are predisposed to micro- and macrovascular complications that result in significant morbidity and mortality [8,9]. They are characterized by low plasma level of both enzymatic and non-enzymatic antioxidant defenses, which make their cells prone to oxidative attack leading to the development of both micro- and macrovascular complications [10]. Epidemiological evidence

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suggests that serum catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), chromium (Cr), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn) are potent antioxidants and may play a protective role in the development of chronic diseases including diabetes, cancers, cardiovascular diseases, and inflammatory diseases [11]. Multiple factors have been associated with increased oxidative stress in DM. These factors include glucose autoxidation that results in the production of free radicals, an increase in protein glycation and a decrease in antioxidant defenses [12]. An enhanced oxidative stress is considered an underlying condition that is responsible for some of the complications of DM [13].

It is expected that this study will stimulate interests, discussion and further studies on the role of antioxidant enzymes and minerals vis-a-vis development of DM complications.

Materials and Methods

Study design

The study was designed as a controlled interventional study in which individual subjects are assigned randomly to one of the competing interventions.

Chemicals and reagents

All chemicals and reagents used were of analytical grades. They were all purchased from BDH Chemicals, UK, Sigma-Aldrich, UK, Thermo Fisher Scientific, Nigeria, and Cayman Chemicals, USA.

Alphabetic

Alphabetic (Antioxidants Formula) is a multi-vitamin and mineral supplement recommended by health professionals for people with diabetes (as stated in the leaflet). In addition to Lutein, Cr, and 20 other vitamins and minerals, Alphabetic also contains antioxidant co-enzyme called Alpha Lipoic Acid (as stated in the leaflet). Alphabetic was purchased from Pharmacy Plus Company, Lagos, Nigeria.

Subjects

A total of 200 subjects were recruited for this study. They include non diabetic (50), diabetic non-supplemented (50), diabetic supplemented with Alphabetic (100) attending the Diabetic Clinic of Department of Medicine, Usmanu Danfoyo University Teaching Hospital Sokoto-Nigeria.

Diabetic subjects supplemented with AlphaBetic

These are one hundred (100) diabetic mellitus subjects supplemented with AN antioxidant rich supplement (Alphabetic); they include children, adolescents, and adults of both genders.

Inclusion criteria:

1. The subjects were selected according to position statements of American Diabetes Association, (2010) for DM diagnosis.
   i. Glycated hemoglobin $HbA_{1c} \geq 6.5\%$.*
   Or
   ii. Fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.*
   Or
   iii. Two-hour plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test.*
   Or
   iv. Random plasma glucose ≥ 200 mg/dl (11.1 mmol/l.) in the presence of symptoms of DM (polyuria, polydipsia, polyphagia, and weight loss).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeated testing.

2. DM subjects supplemented with Alphabetic for at least 1 year.

Exclusion criteria:

1. Subjects with gestational diabetes.
2. DM subjects not supplemented for at least 1 year.
3. History of any other chronic disease such as cancer, HIV/AIDS, thyroid dysfunction, and gastrointestenal insufficiency.

Diabetic subject non-supplemented

These are fifty (50) DM subjects not supplemented with antioxidant rich supplements (Alphabetic); they include children, adolescents, and adults of both sexes.

Inclusion criteria:

1. Subjects with DM according to position statements of the American Diabetes Association in 2010 as stated earlier.2. DM subjects that are not supplemented with any antioxidant drugs.

Exclusion criteria:

1. Subjects with gestational diabetes.
2. History of any other chronic disease such as cancer, HIV/AIDS, thyroid dysfunction, and gastrointestenal insufficiency.

Non-diabetic subjects

Fifty (50) non-diabetic, normal and apparently healthy subjects were recruited.

Inclusion criteria:

1. Normal and apparently healthy individuals.

Exclusion criteria:

1. Individuals who suffer from diabetes, hypertension, cardiovascular disease, and any other chronic disease.
2. Individuals who are under cortisol treatment or suffer from any autoimmune disease.

Sample Collection and Preparation

Five (5) ml of blood were collected in plain containers via venepuncture from all subjects. After collection, samples were centrifuged at 4,000 rpm for 5 minutes, and serum were separated and stored in a deep freeze at −20°C until required for analysis.

Analytical Methods

CAT activity was assayed using chemical reactivity method of Beers and Sizer [14]. The SOD activity was assayed using Cayman's SOD Assay Kit according to the method of Marklund [15]. GPx activity was assayed using Cayman's GPx kit according to the method of Paglia and Valentine [16]. Antioxidant Minerals Levels (Cr, Cu, Mn, Zn, and Se) were estimated by atomic absorption spectroscopy according to the method of Bhatti et al. [17].

Ethical Approval

Approval was obtained from Usmanu Danfodiyo University Teaching Hospital ethical committee. The participants were given a consent form, with an explanation about the purpose of the study. The consent forms were duly signed by participants.

Statistical Analysis

The data collected were expressd as mean ± standard error of the mean. The significance of the differences between groups were determined using one way analysis of variance and Duncan multiple tests were used for comparison, $P < 0.05$ was considered significant. All statistical analyses were done using SPPS software version 21 (IBM Corporation, USA).

Results

Table 1 showed the Serum antioxidant enzyme levels in non-supplemented diabetics, diabetics supplemented with Alphabetic and non-diabetic subjects.

The result revealed that serum activities of CAT and SOD were significantly ($P < 0.05$) lower in diabetic non-supplemented subjects compared to non-diabetic. Supplementation with Alphabetic resulted in the significant increase in the activities of CAT and SOD in the supplemented group compared to the non-supplemented diabetic subjects and slightly higher than non-diabetic subjects ($P < 0.05$). GPx activity was low in non-supplemented diabetic subjects compared to non-diabetic subjects but there was no significant difference between them. Supplementation with Alphabetic resulted in a significant increase [about two (2) times the non-diabetic and three (3) times the diabetic non-supplemented subjects ($P < 0.05$)]. Serum antioxidant minerals levels of DM Non-Supplemented, DM Supplemented with Alphabetic and Non-DM subjects were illustrated in Table 2. Serum levels of Cr and Mn were significantly lower in non-diabetic subjects compared to diabetic non-supplemented subjects ($P < 0.05$). After supplementation with Alphabetic significant increases in Cr and Mn levels were noted when compared to non-diabetic subjects ($P < 0.05$). There was no significant difference in blood Cu levels across all subjects but serum Cu was lower in diabetic supplemented and diabetic non-supplemented than non-diabetic subjects, i.e., before and after supplementation of Alphabetic ($P < 0.05$). There was a significant decrease in

Table 1. Serum antioxidant enzymes activities in diabetic non-supplemented, diabetic supplemented with Alphabetic and non-Diabetic subjects. Values are Mean ± Standard Error of the Mean.

	SOD (U/ml)	GPx (nmol/min/ml)	CAT (U/ml)
DMNS (n = 50)	0.77 ± 0.04^a	19.24 ± 2.08^a	23.55 ± 2.83^a
DMSA (n = 100)	1.01 ± 0.00^b	60.84 ± 5.41^b	45.03 ± 3.03^b
NODM (n = 50)	0.94 ± 0.06^b	24.95 ± 6.18^a	34.62 ± 4.47^b

Values bearing different superscripts on the same column differ significantly ($P < 0.05$). SOD: superoxide dismutase, GPx: glutathione peroxidase, CAT: catalase, n: number of subjects, DMNS: Diabetic non-supplemented, DMSA: Diabetic supplemented with Alphabetic, and NODM: Non-diabetic Mellitus.

Table 2. Serum antioxidant minerals level of diabetic non-supplemented, diabetic supplemented with Alphabetic and non-diabetic subjects. Values are mean ± standard error of the mean.

	Cr (mg/dl)	Cu (mg/dl)	Mn (mg/dl)	Zn (mg/dl)	Se (mg/dl)
DMNS (*n* = 50)	0.09 ± 0.03[b]	0.99 ± 0.09[a]	0.23 ± 0.04[b]	0.04 ± 0.01[a]	0.14 ± 0.02[b]
DMSA (*n* = 100)	1.00 ± 0.08[c]	1.15 ± 0.07[a]	1.69 ± 0.15[c]	1.28 ± 0.08[b]	1.72 ± 0.16[a]
NODM (*n* = 50)	0.35 ± 0.03[a]	1.22 ± 0.11[a]	0.69 ± 0.15[a]	0.90 ± 0.25[b]	0.43 ± 0.04[b]

Values bearing different superscripts on the same column differ significantly ($P < 0.05$). Cr: chromium, Cu: cupper, Mn: manganese, Zn: zinc, and Se: selenium, *n*: number of subjects, DMNS: Diabetic non-supplemented, DMSA: Diabetic supplemented with Alphabetic, and NODM: Non-diabetic Mellitus.

blood Zn levels in diabetic non-supplemented subjects when compared to non-diabetic subjects, after supplementation with antioxidant rich supplement (Alphabetic) the result showed significant ($P < 0.05$) improvement in blood Zn level when compared to diabetic non-supplemented subjects and a mild increase when compared to non-diabetic subjects ($P < 0.05$). The serum Se level was significantly decreased in diabetic non-supplemented subjects when compared to non-diabetic subjects ($P < 0.05$). But after supplementation with Alphabetic, the serum Se was significantly improved ($P < 0.05$).

Table 3 demonstrated the correlation coefficient (r) of serum antioxidant enzymes with serum antioxidant minerals of diabetic supplemented subjects. All parameters showed a positive linear correlation ($P < 0.05$).

A positive correlation, when r is greater than 0, signifies that both variables move in the same direction. When r is +1, it signifies that the two variables being compared have a perfect positive relationship; when one variable moves higher or lower, the other variable moves in the same direction with the same magnitude. The closer the value of r is to +1, the stronger the linear relationship. The results revealed a positive correlation coefficient (r) of serum antioxidant enzymes with antioxidant minerals was recorded. Therefore, it means that as the level of antioxidant minerals increased in diabetic supplemented subjects, the activity of serum antioxidant enzymes will also increase.

Table 3. Correlation coefficient (r) of serum antioxidant enzymes with serum antioxidant minerals of diabetic supplemented with Alphabetic subjects.

	Cr	Mn	Cu	Zn	Se
SOD	0.20	0.46	0.50	0.54	0.28
GPx	0.25	0.19	0.30	0.31	0.52
CAT	0.03	0.36	0.01	0.14	0.25

Cr: Chromium, Mn: Manganese, Cu: Copper, Zn: Zinc, Se: Selenium, SOD: superoxide dismutase, GPx: glutathione peroxidase, and CAT: catalase.

The correlation coefficient (r) of serum antioxidant enzymes with serum antioxidant minerals of non-supplemented diabetic subjects was shown in Table 4. All values showed a positive linear relationship ($P < 0.05$).

The results revealed positive correlation coefficient (r) of serum antioxidant enzymes with antioxidant minerals. Positive correlation means that as the level of antioxidant minerals increases in non-supplemented diabetic subjects, the activity of serum antioxidant enzymes also simultaneously increase as observed in the case diabetic supplemented.

Discussion

Many studies have demonstrated the presence of oxidative stress in DM as a result of increased free radical production and diminished antioxidant defenses.

The significant ($P < 0.05$) increases in the levels of antioxidant enzymes (SOD, CAT and GPx) were observed in all subjects supplemented with Alphabetic compared to non-supplemented diabetic subjects. This could be associated with the presence of cofactors of these enzymes in the Alphabetic supplement such as Se [18].

The GPx activity significantly ($P < 0.05$) increased in supplemented diabetic subjects to compare non-diabetic and non-supplemented diabetic subjects (Table 1). The increase might be due to the increased availability of Se which is an important co-factor for GPx [18]. These findings are in agreement with other previous findings [19,20] in which an increase in the activity GPx was observed and decreased concentration of Malondialdehyde (MDA) in diabetic subjects supplemented with the mixture of antioxidant vitamins and trace minerals was reported.

CAT activity also significantly increased in supplemented diabetic subjects and non-diabetic subjects compared to non-supplemented diabetic

Table 4. Correlation coefficient (*r*) of serum antioxidant enzymes with serum antioxidant minerals of non-supplemented diabetic subjects.

	Cr	Mn	Cu	Zn	Se
SOD	0.18	0.36	0.42	0.39	0.09
GPx	0.27	0.29	0.27	0.14	0.54
CAT	0.28	0.33	0.32	0.28	0.25

Cr: Chromium, Mn: Manganese, Cu: Copper, Zn: Zinc, Se: Selenium, SOD: superoxide dismutase, GPx: glutathione peroxidase, and CAT: catalase.

subjects ($P < 0.05$). CAT is believed to play a role in cellular antioxidant defense mechanisms by limiting the accumulation of hydrogen peroxide (H_2O_2). It functions to catalyze the decomposition of hydrogen peroxide to water and oxygen [21]. Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances [22]. Therefore, CAT is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Similar findings were early reported [23].

SOD activity significantly decreases in non-supplemented diabetic subjects compared to diabetic supplemented and non-diabetic subjects indicating oxidative stress in diabetes ($P < 0.05$). The improvement in its activity upon supplementation with Alphabetic could be due to the presence, and Zn and Cu are important cofactors for SOD. SOD is the most effective intracellular enzymatic antioxidants and it catalyzes the conversion of superoxide anions to dioxygen (O_2) and hydrogen peroxide. SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity [22]. In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly [18]. SOD is necessary because superoxide reacts with sensitive and critical cellular targets. For example, it reacts with the NO radical and makes toxic peroxynitrite. The findings are in agreement with the report [23,24].

The results of this current work indicated that serum concentrations of Cr, Mn, Zn, and Se were significantly ($P < 0.05$) lower in non-supplemented diabetic subjects when compared to non-diabetic and supplemented diabetic subjects. In addition, diabetic supplemented subjects had higher blood levels of Se, Zn, Mn, and Cr when compared to diabetic non-supplemented subjects. Increased oxidative stress in diabetic subjects resulted in higher utilization of these minerals and consequently their deficiencies. However, supplementation with Alphabetic that is rich in these antioxidant minerals may help to restore the balance of these antioxidant minerals.

The implication of these findings could not be over emphasized. Alphabetic has been reported to have components that increase insulin binding to cells, number of insulin receptors, and activates insulin receptor kinase leading to increased insulin sensitivity [25]. Accordingly, severe Cr deficiency was implicated as a cause of impaired glucose tolerance and subsequent hyperglycemia and glucosuria [25]. Cr was reported to raise plasma high density lipoprotein: low density lipoprotein ratio [26]. Mn has been found to be important in insulin synthesis and secretion. It has been shown that type 2 diabetic subjects responded well to oral doses of Mn. There were conflicting reports of manganese deficiency in DM [27]. The increased urinary loss of Zn is a commonly encountered feature of diabetes. High doses of oral Zn might enhance wound healing [26]. There was no significant ($P < 0.05$) difference in Cu concentration across all the groups (Table 2). There is a significant increase of Se in supplemented diabetic subjects when compared to non-diabetic subjects and a significant decrease was noted in non-supplemented diabetic subjects compared to non-diabetic subjects. Se is a component of enzymes that catalyze redox reactions and it acts as an antioxidant in the form of selenoproteins which contain selenocysteine [28]. The best known selenoproteins are GPx. Reduced Se levels have been observed in diabetic non-supplemented subjects together with increased oxidative stress [29,30]. Those observations are in the same direction with those of [31,32] who studied the antioxidant and anti-inflammatory effects of Se and thus supplementation may be considered in subjects with diabetes due to the role played by oxidative stress and inflammation in these conditions. Further support to our findings are the reports of [20,33] who compared blood levels of antioxidants minerals and Vitamins in Sokoto and Katsina.

The current results revealed a positive correlation between antioxidants, enzymes, and minerals (the level of antioxidant minerals increases then the activity of antioxidant enzymes will also simultaneously increase). This is because the antioxidant vitamins require the antioxidant minerals as cofactors

for their function and therefore is not surprising that a positive correlation was observed in both diabetic supplemented and non-supplemented subjects [20]. This also indicated that minerals may function as regulators of antioxidant enzymes, thus their deficiency may negatively affect the cellular antioxidant enzyme defense system.

Numerous studies have recorded the alterations in micronutrients status of patients with DM. In some studies, deficiency of certain minerals and vitamins has been correlated with the presence of diabetic complications such as retinopathy, neuropathy, nephropathy, atherosclerosis, etc. Quite a number of antioxidants participate in the protection of human body against free radical pathology and its consequences [24].

Conclusion

The current work reported that supplementation of diabetic subjects with antioxidant rich supplement (Alphabetic), significantly reverse oxidative stress in diabetic subjects. The results indicated that the supplementation increased Se, Zn, Mn, Cr as well as the activity of antioxidant enzymes (SOD, CAT, and GPx). Based on these findings, it could be concluded that supplementation with antioxidants rich supplements (e.g., Alphabetic) might reverse the risk of oxidative stress associated with diabetes that lead to development of diabetic complications. The major limitation of the study is inability to get matched number control subjects with diabetes for better comparison.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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