

Effects of Selenium Yeast on Blood Glucose and Antioxidant Biomarkers in Cholesterol Fed Diet Induced Type 2 Diabetes Mellitus in Wistar Rats.

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Summary: Selenium is an antioxidant that prevents oxygen radical from damaging cells from chronic diseases that can develop from cell injury and inflammation such as diabetes mellitus. The aim of the study is to investigate the possible protective effect of selenium yeast on cholesterol diet induced type-2 diabetes mellitus and oxidative stress in rats. Twenty male wistar rats were divided in to four groups of five animals each: Group 1: (Negative control) received standard animal feed only, Group 2: received cholesterol diet (CD) only, Group 3: received CD and 0.1 mg/kg selenium yeast orally, Group 4: Received CD and 0.2 mg/kg selenium yeast orally for six weeks. At the end of the study period, the animals were sacrificed and the serum samples were collected and evaluated for estimation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The results showed a significant ($P < 0.05$) decrease in blood glucose level in the groups co-administered CD and selenium yeast when compared to CD group only. Antioxidant enzymes status recorded significant ($P < 0.05$) decrease in SOD, CAT and GPx activities in CD and selenium yeast administered when compared to CD group only. In Conclusion, Selenium yeast administrations prevent free radical formations which are potent inducer of diabetes mellitus.

Keywords: Cholesterol diet; Diabetes Mellitus; Selenium yeast; SOD; CAT; GPx

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INTRODUCTION

Selenium yeast is a recognised source of organic nutrient from selenium and naturally present in various food types such as Brazil nuts, chicken, fish, turkey, crab, nuts, cereal and eggs. It was discovered in 1817 and reported in 1818 by Jon's Jacob Berzelius (Arner, 2010). Selenium is a dietary supplement and micronutrient in humans, which is well known for having an extremely thin border between beneficial and toxic concentrations. It is the only trace element to be specific in the genetic code as selenocysteine (Meneilly and Tessier, 2001). Selenium can exist in both organic (as selenomethionine, selenocysteine, and selenoproteins) and inorganic (as selenite and selenates). The role of selenium in prevention of chronic diseases is undoubtedly an aspect where intense investigations are being made. Primarily, most research on selenium is mainly on evaluating the potential benefits of its antioxidant and anticancer effect (Navas-Acien *et al.*, 2008; Rayman, 2008). Selenium is physiologically essential and may also offer a protective effect against several degenerative diseases (Navaro-Alarcon and Lopez-Martinez, 2000).

The organic form of selenium provided by selenium yeast has been shown to differ in bioavailability and metabolism compared with inorganic (e.g., selenate, selenite) forms of dietary selenium (Schrauzer, 2000). Selenium yeast has been used in a wide range of studies aimed at examining the importance of selenium status in the incidence and progression of a variety of infectious and degenerative diseases (Lovell *et al.*, 2009).

Diabetes mellitus Type 2 (formerly noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by hyperglycemia (high blood sugar) in the context of insulin resistance and relative lack of insulin (Kumar *et al.*, 2005). Development of Type 2 diabetes is caused by a combination of lifestyle, genetic, increasing age, female gender (Ripsin *et al.*, 2009; Risérus *et al.*, 2009). Two clinical constructs for identifying individuals at high risk of developing Type 2 diabetes are prediabetes and metabolic syndrome (MetS). Individuals with MetS are at a five-fold increased risk of developing Type 2 diabetes (Alberti *et al.*, 2009). Prediabetes is a state of dysglycemia defined by impaired fasting glucose (IFG) and/or

impaired glucose tolerance (American Diabetes Association, 2012). Over consumption of high fat diet result has been reported to cause accumulation of fat in adipose tissue which consequently resulted into dysregulation of lipid metabolism that cause Type 2 diabetes mellitus (Jimoh *et al.*, 2015). Type 2 diabetes mellitus is a potent inducer of reactive oxygen species and formation of oxidative stress (Youn *et al.*, 2014). In recent years, diabetes has become one of the often occurring metabolic diseases in developed countries. Since untreated diabetes predisposes to oxidative stress, antioxidant supplements were considered to be favourable.

The aim of the study is to investigate the possible protective effect of selenium yeast on cholesterol diet induced type-2 diabetes mellitus and oxidative stress in rats.

MATERIALS AND METHODS

Equipment

Digital glucometer (Accu-check advantage, Boche Diagnostic, Company); weighing balance (GF 2000), syringes and needles (Sologuard Medical Device P.V.T Ltd., Chema-600 096, India, ML No. 750).

Chemical Used

All chemicals were obtained commercially and were of analytical grade: (Cholesterol: Sigma chemical Company St. Louis USA) and Selenium-yeast (Sigma-Aldrich).

Animals

Wistar rats of both sexes weighing 70 g – 100 g bred in the Animal House, Department of Human Physiology, Ahmadu Bello University, Zaria were used for the study. The animals were kept in well-aerated laboratory cages in the Departmental Animal House, and were allowed to adjust to the laboratory conditions for a period of two weeks before the commencement of the experiment. They were fed with growers and starters mash from Vital Feeds Company Kaduna State, Nigeria, and water were provided during the stabilizing period. The study was conducted in accordance with the guidelines of Ahmadu Bello University rules governing the use of laboratory animals as accepted internationally by (National Institute of Health Guide for Care and Use of Laboratory Animals).

Experimental design

In the study, twenty (20) Wistar rats weighing between 70g-100g were used for the study. The rats were randomly divided into 4 groups of five (n = 5) animals in each

Group I: Normoglycaemic control were given normal feed only

Group II: Diabetic control untreated was given Cholesterol diet only (CD) for a period of six weeks.

Group III: CD + Selenium yeast (S.Y) 0.1 mg/kg for a period of six week.

Group IV: CD + Selenium yeast (S.Y) 0.2 mg/kg (Vikas *et al.*, 2013) for a period of six weeks.

Induction of diabetes mellitus and oxidative stress

The animals were fasted for 16-18 hours before the commencement of the experiment, but were allowed water *ad libitum*. The normal groups were fed with standard animal feeds only; while the animals were fed with high fat diet made of cholesterol diet (10 % Groundnut oil, 20 % Groundnut mill and 1% cholesterol/kg/day) for the induction of diabetes mellitus and oxidative stress for 6 weeks of experimental period (Kolawole *et al.*, 2012) with slight modification.

Determination of Blood Glucose Level

Blood samples (5mls) were collected by cutting the tail of the rats weekly for a period of 6 weeks. Blood glucose level was determined by glucose oxidase method by using a Digital Glucometer (Accu-Check Advantage, Roche Diagnostic, Germany), and results were obtained as mg/dL (Rheney and Kirh, 2000).

Collection and Preparation of Serum Samples for Analysis

6 weeks after the treatment period, all animals were subjected to light anesthesia by exposing them to chloroform soaked in cotton wool placed in anaesthetic box covered with lid. Blood samples of about 3ml were drawn from the heart of each sacrificed animal from all groups by cardiac puncture. The samples were collected in Eppendorf tubes and were allowed to clot. Thereafter the serum was separated by centrifugation, using Denley BS400 centrifuge (England) at 3000 g for 10 minutes. The supernatant collected were used for analysis of oxidative stress biomarkers.

Antioxidant Enzymes assay:

Superoxide Dismutase Activity

Activity of SOD in the rabbit serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The assay kit is based on the principle of superoxide inhibition of autooxidant of hematoxylin as described by Martin *et al.* (1987).

Catalase Activity

CAT activity was assessed using NWLSS™ CAT activity assay kit (Product NWK-CAT01, Specificity: Catalase, Sensitivity: 6.0 U Catalase/mL). Catalase enzyme activity was measured based on the principle of catalase consumption of H₂O₂ substrate at 240 nm (Beers and Sizer, 1952).

Glutathione Peroxidase Activity

GPx activity was assessed using NWLSS™ cGPx (GPx1) ELISA assay kit (Product NWK-GPx02, Specificity: Glutathione peroxidase, Sensitivity: 12.5pg/ml). The NWLSS™ cGPx Assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA), where sample GPx concentration is

determined by comparing the 450nm absorbance of sample wells to the absorbance of known standards (Takebe, 2002).

Statistical analysis

Data obtained were expressed in kg as mean ± SEM. The data were analyzed using ANOVA followed by Dunnett's *post-hoc* test to show multiple comparisons versus control group. Values of P < 0.05 were considered as significant (Duncan *et al.*, 1977).

RESULTS

Effects of Selenium Yeast on Blood Glucose in Cholesterol Fed Diet Induced Type 2 Diabetes Mellitus in Wistar Rats for a period of six weeks.

Table 1 shows the results of the effects of two doses (0.1 mg/kg and 0.2 mg/kg) of selenium yeast with cholesterol diet in rats, normal fed rats and cholesterol diet group only. At four weeks of the experiment, 0.1 mg/kg and 0.2 mg/kg selenium yeast administered recorded a significant (P < 0.05) decrease in blood glucose level with respective values of 78.20 ± 4.47 mg/dL and 68.20 ± 5.57 mg/dL when compared with a value of 120.20 ± 3.57 mg/dL for cholesterol diet.

Antioxidant enzyme assay:

Superoxide dismutase

Figure 1 shows the activity of SOD in the selenium yeast administered with CD, control group alone and CD group only. Selenium yeast co-administered with cholesterol diet showed significant (p < 0.05) decrease in SOD activity of 0.1 mg/kg and 0.2 mg/kg selenium yeast with values of 49.60 ± 1.60 IU/L and 48.20 ± 1.36 IU/L when compared to the CD group only with a value of 67.40 ± 2.48 IU/L respectively. The result shows that the activity of SOD was decreased despite consumption of CD in groups treated with selenium yeast.

Glutathione peroxidase activity

Figure 2 shows the activity of GPx in the selenium yeast administered with CD and CD group only. Selenium yeast co-administered with CD recorded a significant (p < 0.05) decrease in GPx activity with a value of 42.60 ± 2.73 IU/L for 0.1 mg/kg selenium yeast and 43.40 ± 1.08 IU/L for 0.2 mg/kg selenium yeast when compared to the CD group only with a value of 58.40 ± 1.21 IU/L respectively. Selenium

yeast administration ameliorates GPx activity despite consumption of cholesterol diet.

Catalase activity

CAT activities in the selenium yeast co-administered with CD, control group alone and CD group only are shown in figure 3. 0.1 mg/kg and 0.2 mg/kg selenium yeast co-administered with CD diet showed significant (p < 0.05) decrease in CAT activity with values of 1.12 ± 0.16 IU/L and 1.14 ± 0.16 IU/L when compared to the cholesterol diet group only with a value of 2.50 ± 0.15 respectively.

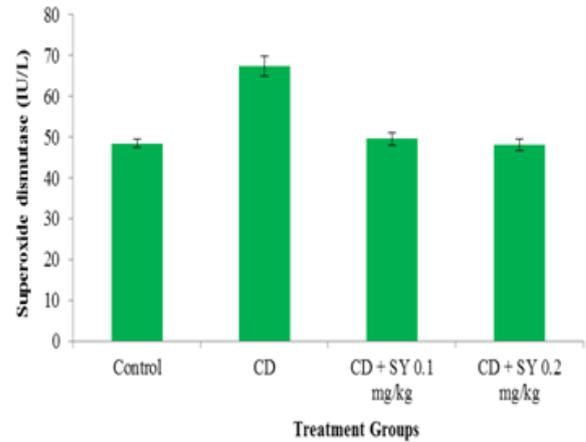


Figure 1: Effect of co-administration of selenium and high-fat diet on SOD activity in rabbits as compared with fed and unfed control group. Values are expressed as mean ± SEM; n = 5. ^a = p < 0.05 significant as compared to diabetic control untreated (CD).

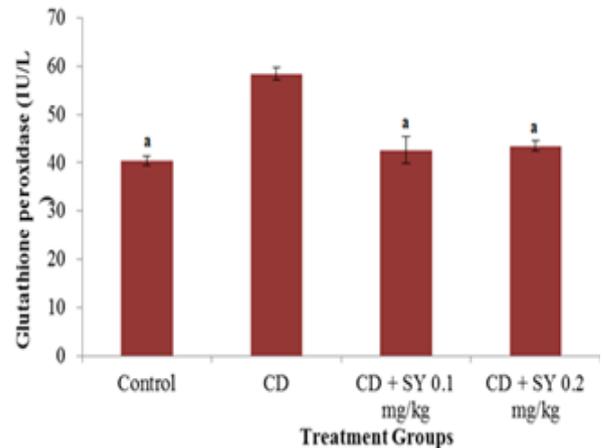


Figure 2: Effect of co-administration of selenium and high-fat diet on serum GPx level in rabbits as compared with fed and unfed control group. Values are expressed as mean ± SEM; n = 5. ^a = p < 0.05 significant as compared to diabetic control untreated (CD).

Table 1: Effects of Selenium Yeast on Blood Glucose in Cholesterol Fed Diet Induced Type 2 Diabetes Mellitus in Wistar Rats for a period of six weeks.

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	109.60 ± 2.98	100.20 ± 4.28	102.40 ± 6.55	112.60 ± 6.40	107.40 ± 5.22	98.00 ± 2.82 ^a	98.40 ± 3.01 ^a
CD	98.20 ± 3.61	99.00 ± 10.82	107.80 ± 2.63	95.40 ± 21.13	120.20 ± 3.57	129.20 ± 4.24	138.00 ± 6.40
CD + SY (0.1 mg/kg)	98.20 ± 2.48	91.80 ± 2.59	119.00 ± 3.03	97.00 ± 3.74	78.20 ± 4.47 ^a	94.40 ± 4.02 ^a	84.20 ± 8.23 ^a
CD + SY + 0.2mg/kg	99.00 ± 3.11	84.20 ± 4.76	104.00 ± 6.43	108.00 ± 4.22	68.20 ± 5.57 ^a	87.20 ± 5.98 ^a	96.80 ± 1.43 ^a

^a = p < 0.05

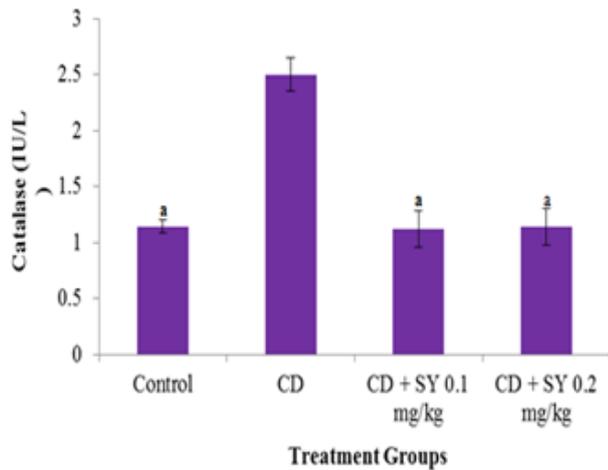


Figure 3: Effect of co-administration of selenium and high-fat diet on serum level of CAT in rabbits as compared with fed and unfed control group. Values are expressed as mean \pm SEM; n = 5. ^a = p < 0.05 significant as compared to diabetic control untreated (CD).

DISCUSSION

The use of selenium yeast (Se-Y) as enriched selenium supplements in human nutrition has developed a topic of cumulative interest over the last decade. Experimental studies suggest that antioxidant supplements such as selenium, at the nutritional level, could delay the development of diabetes by decreasing oxidative stress (Steinbrenner *et al.*, 2009). Consumptions of cholesterol diet in excess has been known to cause lipidaemia and subsequently type-2 diabetes mellitus in laboratory animals (Kolawole *et al.* 2012; Jimoh *et al.*, 2015).

The result of the present study showed that plasma blood glucose level in the group treated with selenium yeast was significantly lowered compared to the cholesterol group only. This result agrees with the finding of Mohammed *et al.* (2015), who demonstrated that selenium yeast decreases blood glucose level in STZ induced diabetes in wistar rats. The present result disagrees with the work of Park *et al.* (2012), who demonstrated that individuals consuming a normal diet with the highest toenail selenium amounts were discovered to be at a very low risk for type 2 diabetes mellitus. The decreased or increased in blood glucose level may be possible that the relationship is U-shaped, with possible harm occurring both below and above the physiological range for optimal activity of some or all selenoproteins (Rayman and Stranges, 2013). Another study reported that diabetic men have lower levels of toenail selenium than that of non-diabetic individuals acting as controls (Rajpathak *et al.*, 2005). Interestingly, individuals consuming a normal diet with the highest toenail selenium amounts were discovered to be at a very low risk for type 2 diabetes mellitus (Park *et al.*, 2012). Based on these contrasting reports, in correlation to the pervasiveness of type 2 diabetes, it is imperative to examine the potential benefits and harms of dietary selenium supplementation on the aetiology of type 2 diabetes

mellitus. Amongst its potential benefits, adults with relatively low selenium status did not develop any diabetogenic effects after six months supplementation (Rayman, 2000). These corroborating epidemiological reports suggest that absolutely high selenium in plasma, not selenium itself, may be the reason for the increased risk found in those with adequate selenium status and supplementation. Furthermore, taking selenium supplement on top of an adequate dietary intake increases the risk of diabetes. Conclusively, these suggestions above, clarify that selenium deficiency and a high expression of selenium in serum plasma, predisposes to diabetogenic effects (Bleys *et al.*, 2007).

Though the mechanism has not yet been fully elucidated. But according to previous reports, it is with the aid of selenoproteins, that selenium carries out specific biological functions like protection against oxidative stress, thyroid function and immune functions (Rayman, 2000). The significant decrease in antioxidant status results observed in group treated with selenium yeast as compared to untreated group indicates that selenium yeast offers protection against increase in reactive oxygen species in our studies. The increase in antioxidant enzymes in CD group may represent compensatory vicissitudes in response to proliferation of ROS which if not managed, results in to health impediments. These results correlate to the work of Calamari *et al.* (2010) in which he determined the effects of dietary Se source and dose on metabolic and haematological profiles, and their relationships with oxidative status in horses. Increase in these ROS in cholesterol group may in part explain the observed increase in blood glucose level in this same group. The antioxidant enzymes play a key role in the cellular defence against ROS (Bernabucci *et al.*, 2002). Superoxide dismutase (SOD) serves as the first line of defence against the deleterious effect of ROS. The function of intracellular SOD is to scavenge superoxide (O_2^-) produced by cellular metabolism, and catalyse dismutation of superoxide to oxygen (O_2) and hydrogen peroxide (H_2O_2) (Bernabucci *et al.*, 2002; Das, 2011). The increase dismutation of O_2^- by SOD leads to increase in production of H_2O_2 , which is further detoxified by CAT and GPx to H_2O and O_2 (Das, 2011). According to current literatures, there are various studies reporting that diabetic subjects tend to have more oxidative internal environments than healthy normal subjects (Bloch-Damti and Basham, 2005; Phillips *et al.*, 2004). From these studies it can be deduced that diabetic subjects show an increase in ROS generation and oxidative stress markers which is usually accompanied by decrease in antioxidant levels in the body. The possible sources of oxidative stress in diabetes might include auto-oxidation of glucose, decreased tissue concentrations of low molecular weight antioxidants, such as reduced glutathione (GSH) and ascorbic acid, and impaired activities of antioxidant defence enzymes such as superoxide

dismutase (SOD) and catalase (CAT) (Haskins *et al.*, 2003). Hyperglycaemia, a common factor for type 2 diabetics, is a major contributor to oxidative stress. However, the elevated extra- and intra-cellular glucose concentrations, which brings about the onset of oxidative stress (West, 2000) was reported both in experimental diabetes in animals and in diabetic patients. This hyperglycaemic condition has been associated with the onset of type 2 diabetes (T2DM) via insulin resistance (Sayed *et al.*, 2011; Agnieszka *et al.*, 2011).

Cholesterol diet induced oxidative stress in rats, resulting in increased levels of ROS and subsequently hyperglycaemia. Co-administration of selenium yeast mitigates some compensatory and endogenous defence mechanisms which serve as a protection against ROS and hyperglycaemia.

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