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Research Article

Zinc-Supplemented Diet Ameliorates Renal Lesion with Concomitant Reduction in Body Mass Index and Enhanced Glycemic Control in Experimental Diabetic Rats

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Abstract

Hyperzincuria associated with frequent polyuria in an uncontrolled diabetes mellitus have been reported to contribute significantly to poor glycemic control and profile in diabetics. This experimentally-controlled designed nutritional study, aimed to determine the effects of Zn-supplemented diet on glycemic profile, tolerance and control, body mass index and renal histoarchitecture in diabetic male rats. Twenty four male Wistar rats each weighing ≥ 200g were randomly categorized into three experimental groups (n = 8, each): Normal control fed with standard diet (CN); Diabetic control fed with standard diet (CD) and Diabetic on zinc-supplemented diet (ZD). Diabetes was inducted with freshly prepared alloxan monohydrate solution (150 mg/dL, intraperitoneally). Rats were fed for a period of eight weeks according to the experimental design with water *ad libitum* while their anthropometric measurements were taken twice weekly to determine body mass index (BMI). Fasting blood sugar (FBS) levels were measured twice weekly while oral glucose tolerance test (OGTT) conducted at the end of study. Animals were sacrificed at the end of eight week to extract kidneys for histomorphometric analysis. Microsoft Excel with statistical program SPSS version 22.0 and Graph Pad Prism were used to analyse data while P values < 0.05 were considered significant. Zn-supplemented diet caused significant ($p < 0.05$) reductions in mean body weight gain (11.7 %) and BMI (6.6%), ameliorated renal lesion evidenced by improved renal histophotomicrograph and improved glycemic tolerance and control. In conclusion, zinc-supplemented diet ameliorated renal lesions with concomitant reduction in body mass index and enhanced glycemic control in male diabetic rats.

Keywords: *Body Mass Index; Diabetic Rats; Glycemic Control; Renal Histoarchitecture; Zinc-Supplemented Diet*

Introduction

Dietary supplementation of zinc in diabetics has been proved to improve life quality and expectancy as reported by both animal [1,2] and human [3-7] studies. Zinc insufficiency has been recognized by a number of experts as an important public health issue especially in developing countries [8]. Previous studies indicated that marginal zinc deficiency is more prevalent among diabetic adults compared to the normal adult population [9] while abnormal zinc plasma levels occur more frequently in metabolically uncontrolled diabetic patients [10]. Relationship between zinc and leptin levels has been investigated in previous study [11] while the correlation between zinc supplementation and body mass index was determined in human subjects [12]. The kidney is rich in zinc-containing enzymes which played

significant role in kidney functions. Hyperzincuria associated with frequent polyuria in an uncontrolled diabetes mellitus has been reported to contribute significantly to poor glycemic control and profile in diabetics [13]. Thus, supplementing this important trace element in dietary menu of diabetics as a form of replacement therapy may as well minimize the associated problems with zinc deficiency while concomitantly improving the glycemic tolerance and control. This experimentally-controlled designed nutritional study therefore, aimed to determine the effects of Zn-supplemented diet on glycemic profile, tolerance and control, body mass index and renal histoarchitecture in diabetic male rats.

Materials and Methods

Experimental animals and design

Twenty four (24) adult male Wistar rats (*Rattus norvegicus*) weighing ≥ 200g were purchased from the disease-free stock of the animal house of the Faculty of Basic Medical Sciences, University of Medical Sciences Ondo, Ondo State Nigeria. They were fed initially with standard rat chow and water *ad libitum* for the 2 weeks acclimatization in raised stainless steel cages with 6mm^2 mesh floor (to maintain same physical activity) kept in a well-ventilated animal house maintained under standard conditions (12:12h light:dark cycle; 25 ± 2°C, relative humidity). Replaceable numbered blotters papers were placed under each cage to catch the spilled diet that was measured to make up for the daily serving ration. After acclimatization, the rats were randomly divided into three groups of 8 rats each: Normal control (CN) fed with standard rat feed; Diabetic control (CD) fed with control diet and Diabetic fed on Zn-supplemented diet (ZD). Each group had a close entry value of mean body weight (Table 2) and coefficient of variation. All animal weights were measured twice weekly and recorded. This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care [14] with the approval of the Animal Care and Use Review Committee of the Institution.

Diets composition and feeding

The composition of the diets in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The control (normal ration) and the test (Zn-supplemented ration) diets were prepared from ingredients purchased from a commercial market in Ibadan metropolis, Oyo State, Nigeria according to the compositions (expressed in percentage per 100g feed) shown in table 1. Both control and test diets contain same calories while zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) from Koch-light laboratories Ltd Colinbrook Berks England was used as the source of the zinc which was mixed with the control diet to form the test diet. The amount of zinc used in the test diets for daily serving size was based on the total weight of the number of rats per group equivalent to 50 mg $ZnSO_4.7H_2O$ per kg diet. The animals were fed according to the experimental design for 8 weeks with water *ad libitum*. Body weight and total food intake of each group of rats were measured and recorded weekly while the food conversion ratio (food intake/weight gain) was calculated.

Control Diet (%)	Nutrient Components	Ingredients	Test Diet (%)
40	Carbohydrate	Maize	40
15	Fibre	Wheat offal	15
10.5	Protein	Soya bean meal	10.5
20	Fats and Oils	Palm kernel cake	20
10		Groundnut cake	10
0.25		Vitamins B, C, D	0.25
1.0	Minerals	Oyster shell	1.0
3.0		Bone meal	3.0
0.2	Amino Acids	Methionine, Lysine	0.2
-	Supplement (trace element)	Zinc Sulphate ($ZnSO_4$)	0.005
2337.45	Metabolizable Energy kcal/kg)		2337.45
18.58	Crude protein (%)		18.58

Table 1: *Composition of Control and Test Diets (%/100g feed).*

Induction of diabetes

After 15 hour overnight fast following acclimatization, rats in CD and ZD groups were injected by single intraperitoneal injection of 150 mg/kg body weight of freshly prepared 2% Alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile 0.9% normal saline in a standard volumetric flask strapped with foil to prevent alloxan instability. Diabetes was confirmed 4-7 days later by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips. Rats with Fasting Blood Glucose (FBG) level > 150 mg/dl were considered diabetic and used for this study since the level of serum glucose considered to be normal in *Rattus norvegicus* ranges from 50 - 135 mg/dL [15]. Diabetes was allowed to stabilize for 5 days before animal grouping and exposure to test diets. Fasting blood glucose levels of all rats in each experimental group were measured on weekly basis for the eight week study period.

Anthropometric parameters measurement

Anthropometric parameters (body weight, length and waist circumference) of all the rats were measured twice a week according to standard methods over a period of eight weeks. Weight were measured to the nearest gram using automated weighing balance while the length and waist circumference expressed in centimetres were measured by using a measuring tape beginning from the nose to the anus and around the waist at hip region above the iliac crest respectively. Values obtained were used to calculate the body mass index (BMI) expressed as weight (g)/ square of length (cm^2).

Blood collections and biochemical assays

Glucometric assay

Blood samples collected from the cordal veins of the rats were used to determine the fasting blood glucose levels of all rats in each experimental group twice on weekly basis for the eight week study period by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips.

Oral glucose tolerance test (OGTT)

Animals in all groups were fasted overnight with free access to water before the last day of eight-week and were administered oral D-glucose load of 2g kg^{-1} (dissolved in distilled water) by means of cannula after taking the initial fasting blood glucose (FBG) concentration. Thereafter, blood samples were withdrawn from the tail vein of each animal (tail snipping) to determine the fasting blood sugar concentration at intervals of 30, 60, 90 and 120 minutes using glucose analyzer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.).

Kidney extraction and histological analysis

At the end of the study, animals in all groups were anesthetized using Ethyl Ether in a glass dome and then dissected to extract the kidneys for histological studies. Tissues were histologically processed using standard laboratory histotechniques. Extracted organs were placed in 10% formalin solution for a day. All samples were then dehydrated in graded ethanol series, cleared in toluene and embedded in paraffin wax; 5 - 6 µm sections were routinely stained with Harris hematoxylin and eosins stains (Sigma-Aldrich) and were assessed under light microscope (Nikon Eclipse E400).

Statistical analysis

The data obtained were computed, analyzed and summarized using Graph Pad Prism and SPSS program version 22. Results (all mean values) are expressed as mean ± SEM. Comparisons between groups were made using Students t-test and one way analysis of variance (ANOVA). P - values < 0.05 were considered statistically significant.

Results

Effect of zinc-supplemented diet on body anthropometric measures

Body weight and weight gain

The effect of Zn-supplemented diet on mean body weights is presented in table 2. Overall percentage weight gain after 8 weeks was significantly ($P < 0.05$) reduced in ZD rats compared with CD and CN rats as suggested by standard ANOVA. No significant ($P > 0.05$) difference in total food intake and food conversion ratio observed between experimental groups.

Parameters	Experimental Animal Categories		
	Normal Control CN	Diabetic Control CD	Diabetic Zn-Treated ZD
Initial Weight (g)	210.02 ± 1.19	208.14 ± 1.42	212.12 ± 1.10
Final Weight (g)	243.12 ± 2.20	258.10 ± 1.60	187.30 ± 0.18
Weight change (%)	15.65	24.09	-11.70*

Table 2: *Effect of Zinc-supplemented Diet on Body Weights (n = 8/group).*

*Values are expressed in mean ± SEM, *Significant (P < 0.05) when compared with diabetic control - CD.*

Length and waist circumferences

A significant ($p < 0.05$) decrease in waist circumference without significant alteration in body length was observed in zinc-treated rats compared with the diabetic control (Table 3).

	Experimental Animal Categories		
Parameters	Non-diabetic	Diabetic	
	CN	CD	ZD
Mean Body Length (cm)			
Initial	20.12 ± 0.14	20.40 ± 0.04	20.10 ± 0.12
Final	20.16 ± 1.12	20.42 ± 0.26	20.12 ± 0.21
% Change in Length	0.20	0.10	0.10
Mean Waist Circumference (cm)			
Initial	10.14 ± 0.15	10.01 ± 0.22	10.20 ± 0.11
Final	14.50 ± 1.12	14.20 ± 0.38	11.80 ± 1.38
% Change in Waist Circumference	43.00	41.86	15.69*

Table 3: *Effect of Zinc-supplemented Diet on Length and Waist Circumference (n = 8/group).*

*Values are expressed in mean ± SEM, *Significant (p < 0.05) when compared with diabetic control - CD.*

Body mass index (BMI)

Table 4 depicts the impact of zinc-supplemented diet on BMI (g/cm^2). A significant ($p < 0.05$) reduction in mean BMI was observed in diabetic rats fed with zinc-supplemented diet compared with the diabetic control.

Parameters	Experimental Animal Categories		
	Normal Control CN	Diabetic Control CD	Diabetic Zn-Treated ZD
Initial mean BMI (g/cm^2)	0.60 ± 0.14	0.62 ± 0.08	0.61 ± 0.01
Final mean BMI (g/cm^2)	0.66 ± 0.02	0.70 ± 0.12	0.65 ± 0.20
% change in mean BMI	10.0	12.9	6.6*

Table 4: *Effect of Zinc-supplemented Diet on BMI (n = 8/group).*

*Values are expressed in mean ± SEM, *Significant (p < 0.05) when compared with diabetic control - CD.*

Effect of zinc-supplemented diet on glycemic tolerance and status

Glycemic status (FBS concentrations)

The hypoglycemic effect of Zn-supplemented diet on venous FBS (mg/dL) in diabetic rats is shown in table 5 below. A significant ($P < 0.05$) reduction (23.01%) in mean FBS concentrations was observed in ZD rats over eight week period. The glycemic status of the zinc-supplemented diet-fed rats by the eight week was observed to fall within the normal range values for *Rattus norvegicus* as shown in the table 5 below.

Parameters	Experimental Animal Categories		
	Normal Control CN	Diabetic Control CD	Diabetic Zn-Treated ZD
Entry mean FBS (mg/dL)	76.40 ± 2.20	229.50 ± 2.20	220.10 ± 10.21
Final mean FBS (mg/dL)	72.20 ± 1.48	212.30 ± 4.01	100.30 ± 19.19
% change in mean FBS	0.06	7.50	54.52*

Table 5: *Effect of zinc-supplemented diet on fasting blood sugar concentrations (n = 8/group)*
*Values are expressed in mean ± SEM, *Significant ($p < 0.05$) when compared with diabetic control - CD.*

Glycemic tolerance (GT)

Effect of Zn-supplemented diet on glycemic tolerance was assessed by the incremental areas under the glycemic response curves as depicted in figure 1 below. Zn-supplemented diet significantly enhanced glycemic tolerance in ZD rats compared with the CD rats.

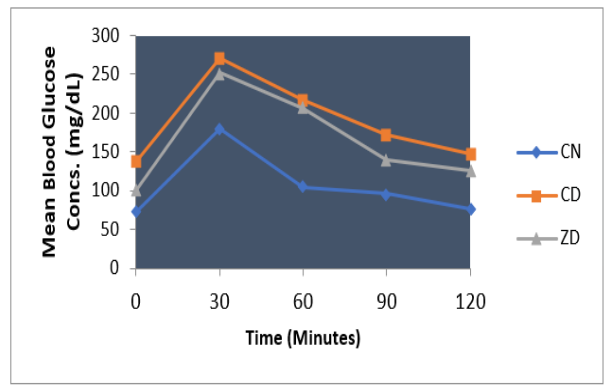

Figure 1: *Glycemic tolerance curves of grouped rats (n = 8/group).*
CD: Diabetic Control group; ZD: Diabetic Fed on Zinc-Supplemented Diet Group; CN: Normal Control Group.

Effect of zinc-supplemented diet on renal histomorphometry

Under high power magnification (x400) light microscopic examination, the photomicrographs (H and E stained) of the renal tissue sections were closely examined. Photomicrograph of the kidney from CN rats (Figure 2) demonstrates normal histoarchitecture while that of the diabetic control (CD) rats (Figure 3) showed mild periglomerular congestion with numerous protein casts in the tubular lamina. Diabetic rats fed with zinc-supplemented diet however, demonstrated no visible lesion with scanty protein casts in tubular lamina (Figure 4).

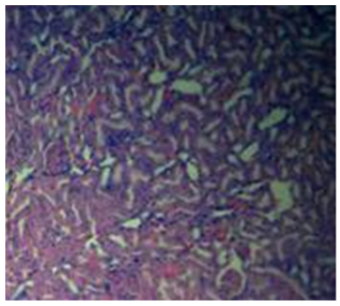

Figure 2: *Renal photomicrograph of CN rats showing normal histoarchitecture.*

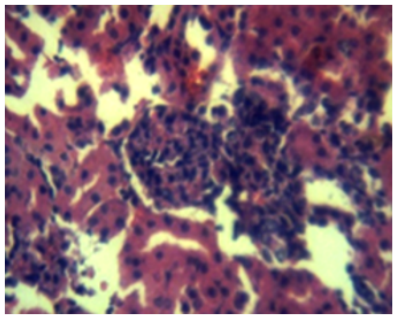

Figure 3: *Renal photomicrograph of CD rats showing mild periglomerular congestion and numerous tubular lumen protein casts.*

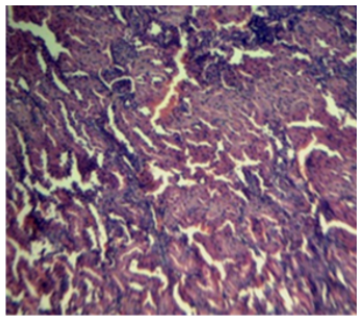

Figure 4: *Renal photomicrograph of ZD rats showing few tubular lumen protein casts with no visible lesion.*

Discussion

The effects of zinc-supplemented diet on body weight gain, renal histomorphometry, body mass index, glycemic tolerance and control in diabetic rats were assessed in this experimentally-controlled designed nutritional study which lasted for eight weeks. Findings obtained revealed that Zn-supplemented diet significantly reduced mean body weight gain, body mass index and fasting blood glucose con-

centrations; ameliorated renal lesions and enhanced glycemic tolerance and control in experimental diabetic male rats. This antidiabetic, antiobesity and renoprotective activities of Zn-supplemented diet displayed its beneficial potentials in dietary control of diabetes mellitus more especially in uncontrolled diabetics in developing countries where the prevalence of zinc deficiency is relatively high.

The composition of the diets used in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The effects of zinc-supplemented diet on body weight gain and body mass index were assessed in diabetic rats. In this study, a significant reduction in mean body weight gain and BMI was observed in diabetic rats fed on Zn-supplemented diet compared with their diabetic control. This observation agrees with the findings of other previous studies using animal [1,16] and human subjects [17] which reported reduction in the observed values of anthropometric parameters such as weight, waist circumference and body mass index. Weight loss is an effective approach in controlling obesity and it has been demonstrated that weight loss improves plasma concentration of glucose, insulin and lipids. Moreover, weight loss has a positive effect on increasing plasma zinc concentration [18]. Possible mechanisms of weight reduction by zinc could arise either from the role of zinc on appetite regulation via leptin system and its receptor through changes in hypothalamic neurotransmitter metabolism [19], preventive role of zinc in the gene mutation which can increase the risk of obesity [20] or similarity of zinc to insulin action in terms of insulin sensitivity and resistance [21].

Zn-supplemented diet in diabetic rats demonstrated beneficial blood glucose lowering effect in this study as it resulted in significant reduction in fasting blood glucose level (54.52%) with improved glycemic tolerance and response as shown by the timed glycemic tolerance profile (Figure 1). This significant ($P < 0.05$) change observed in the supplemented groups reflects the effective improvement in their glycemic control by the zinc-supplemented diet. This finding corresponds with the results of other studies [22,23] that examined the effect of zinc supplementation on patients with type 2 diabetes. Zinc is readily available in animal foods especially lean red meat, beef, liver, fish, and eggs. Based on these findings, diabetics should be encouraged to consume Zn-rich diets (if no contraindication exists) in moderation in order to achieve optimal glycaemic control. Where this is not feasible, then, the prescriptive use of a multivitamin and minerals supplement may be a suitable alternative. Zinc is one of the most important essential trace metals in human nutrition and lifestyle. Its deficiency may severely affect the homeostasis of a biological system.

Effect of zinc on renal histomorphometry was remarkably examined in this study. In the supplemented-grouped renal micrographs, zinc-supplemented diet improved renal histoarchitecture by preventing periglomerular congestion and minimizing accumulation of protein casts in the tubular lamina as shown in figure 4. This finding differs from that observed in the diabetic control rats where numerous protein casts were found in the tubular lamina with mild periglomerular congestion. This observed pathology may be responsible for the associated hyperzincuria due to frequent micturition occurring in uncontrolled diabetes mellitus. Thus, loss of zinc may be corrected or minimized by supplementing the diabetic menu with optimal zinc–rich diet. However, in the course of this recommendation in poorly controlled diabetes, periodical check of blood zinc level should be carried out to prevent hyperzincaemia and its associated toxicity [24].

Conclusion

This experimentally-controlled designed nutritional study revealed the antiobesity, antidiabetic and renoprotective effects of zinc on body mass index, glycemic control and renal histoarchitecture. Therefore, consumption of zinc-rich diets or otherwise, zincs supplements by diabetics should be encouraged for optimal glycemic control.

Conflicts of Interest

No conflict of interest exists.

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