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Antioxidant Supplementation and Kidney Function Status of Wistar Rats Following High Fat Diet-Streptozotocin (HFT-STZ) Induced Type 2 Diabetes

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Abstract

The kidney function status of high fat diet-streptozotocin (HFD-STZ) induced NIDDM in albino rats fed antioxidant supplementation was monitored in vitro. Appropriate and recommended dietary allowed proportions of some potent antioxidant substances including: minerals, vitamins, α-lipoic acid, phytochemicals and a D-ribose-L-cysteine conjugate were assembled together in corn oil and stored at 4°C for use. Kidney function indices were assayed using standard methods, kits and equipments. Data analysis was done with SPSS version 20.0 and significant level was set at $p \leq 0.05$. There were a total of five study groups with 10 rats each. Immediately after the induction of diabetes with HFD-STZ combination, treatment commenced and lasted for a total of 12 weeks, and analysis using serum was carried out at the 4^{th}, 8^{th} and 12^{th} week of the study. Results obtained from the kidney function status investigation indicates that there was significant decrease ($p \leq 0.05$) in serum urea levels of the treated groups when compared to the controls (normal and diabetic) and this decrease was consistent as the treatment progressed. Serum creatinine, biocarbonate and potassium levels of both the treated and normal control groups where not statistically different ($p \geq 0.05$) when compared with the diabetic control group which increased steadily for creatinine and biocarbonate, but inconsistent for potassium level within the treatment duration. However, there was a significant increase ($p \leq 0.05$) in serum sodium and chloride levels of the treated and normal control groups, when compared with the diabetic control group respectively. The observed increase was consistent with treatment duration. The results therefore suggest that the antioxidant supplement might have a restorative effect on kidney function and also enhance effective electrolyte balance and control for easy movement of ions across cell membrane.

Keywords: Biocarbonate; Creatinine; Electrolyes; Urea

Introduction

Diabetes mellitus, a metabolic disease associated with relatively low or absolute deficiency of insulin secretion is the primary cause of diabetic kidney disease (DKD). Diabetic kidney disease progression as one of the critical problems resulting from diabetes mellitus is associated with complications such as retinopathy, neuropathy, hepatopathy, nephropathy and cardiomyopathy, which are primary cause of morbidity and mortality worldwide [1-4]. Hyperglycemia-induced oxidative stress has been reported as one of the links between diabetes and diabetic complications ranging from endothelial dysfunction, insulin resistance, and alterations in the proportion and functions of pancreatic β-cells and ultimately leads to diabetic microvascular and macrovascular complications [5,6]. Hence, glucose oxidation, protein glycosylation and lipid peroxidation are as a result of free radical generation leading to increased reactive oxygen species (ROS). Under physiological conditions, ROS plays an important role in cell signaling implicated in proliferation, differentiation, apoptosis, and immune defense in various cell types, as well as renal cells [7]. However, overproduction of ROS in the kidney under pathological conditions is implicated in renal inflammation, affecting renal structure and function and subsequently leading to end-stage renal disease (ESRD). Chronic renal failure associated with diabetes has increasingly been recognised as a leading source of health concern.

However, oxidative stress associated with free radical generation and reactive oxygen species can be attributed to reduction in antioxidant activity.

Under physiological conditions, both endogenous and exogenous antioxidants interact with these oxidants to counteract the oxidative damage to cells [8]. The antioxidant defense mechanisms include superoxide dismutase (SOD), manganese SOD and copper/zinc SOD; glutathione system: glutathione peroxidase and glutathione reductase; catalase; and coenzyme Q. Antioxidant enzymes mainly convert ROS into nonreactive oxygen molecules, ultimately forming water. The entire antioxidant redox system mainly utilizes NADPH as a chemical reductant, which is mostly produced by glucose-6-phosphate dehydrogenase [9].

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The knowledge and use of key and important antioxidants such as glutathione, vitamin A, C and E in finding modalities for greater understanding on the treatment of diabetic kidney disease and other related complications is vital. Focus on the composition, synthesis and role of glutathione in the understanding of diabetes induced oxidative stress and its complications through different signaling pathways as well as ROS formation attributed to the activation of various downstream signaling cascade affecting structural and functional changes in kidney is of great importance to the management and treatment of diabetic kidney disease and diabetes mellitus in general. A study on the administration of antioxidant agents and its ability to restore the antioxidant defense system thereby preventing ROS mediated injuries is necessary.

Methods

Animal care

Wistar rats aged 3-4 weeks (70-110 g) purchased from the animal house of the Faculty of Pharmacy and relocated to the animal house of the Department of Biochemistry, Madonna University, Nigeria, Elele campus, Rivers State were used for the study. They were housed in well ventilated cages with access to water and food (chow) ad libitum. The animals were grouped into five with 10 rats each in stainless steel cages (34 × 47 × 18 cm) with soft wood shavings as bedding and maintained under normal laboratory conditions (temperature 24-28°C, relative humidity 60-70%, and 12 hour light-dark cycle). All animals used in this study were treated in conformity to the National Institute of Health (NIH) guidelines for handling laboratory animals.

Preparation of high fat diet

The high fat diet (HFD) was prepared according to the method of Srinivasan et al. [10], using growers mash (60 g/kg), lard (20 g/kg) and sucrose (20 g/kg) in the ratio of 3:1:1 respectively. The diet was carefully homogenized and pelleted, then fed to the animals with exception to the normal control group.

Preparation of antioxidant supplement

Recommended proportions of some antioxidant rich substances which include; vitamins A (14.3 mcg/kg bw), B_3 (0.214 mg/kg bw), B_6 (0.03 mg/kg bw), B_{12} (0.03 mg/kg bw), C (0.9 mg/kg bw) and E (0.14 mg/kg bw); minerals like calcium (11.4 mg/kg bw), selenium (0.79 mcg/kg bw), chromium (0.2 mg/kg bw), magnesium (1.9 mg/kg bw), potassium (0.05 g/kg bw) and zinc (0.07 mg/kg bw), α-lipoic acid (8.57 mg/kg bw), cinnamon powder (43 mg/kg bw), curcumin [Meriva®] (3 mg/kg bw), cordyceps (7.5 mg/kg bw), resveratrol (0.5 mg/kg bw), quercetin (2.5 mg/kg bw), D-ribose-L-cysteine [Ribocine®] (30 mg/kg bw) were pulled together in corn oil and stored at 4oC for use.

Induction of type 2 diabetes mellitus

Type 2 diabetes mellitus was experimentally modeled in the animals (with the exception of normal control group) by eight [8] weeks feeding of high fat diet, after which a single dose IP injection of 35mg/kg body weight of streptozotocin was administered.

Treatment

Treatment with standard drug and antioxidant supplement commenced 7 days after fasting blood glucose levels were analysed and hyperglycaemia established (**Table 1**) The treatment lasted for a period of 12 weeks with analysis carried out on the 4^{th}, 8^{th} and 12^{th} week of study.

Table 1 Grouping and feeding illustration.

Group / Treatment key	1	2	3	4	5
No. of rats per group	10	10	10	10	10
Feed + water (Normal control)	+	-	-	-	-
HFD + STZ(35mg/kg) (Diabetic control)	-	+	-	-	-
Diabetes + Standard drug (Actovista)	-	-	+	-	-
Diabetes + AS (antioxidant supplement)	-	-	-	+	-
Diabetes + Standard dietary supplement (Cellgevity) (0.72 mg/kg)	-	-	-	-	+
Key: + : item was administered - : item was not administered					

Assays

The serum urea level was estimated using the method of Weatherburn [11], and serum creatinine level by the method of Bartels and Bohmer [12]. The serum electrolyte levels assayed for were Biocarbonate (HCO_3) by the method of Forrester et al. [13], Potassium (K^+) and sodium (Na^+) were by the method of Henry [14], and chloride (Cl^-) was determined according to the method of Tietz [15].

Statistical Analysis

Data obtained from the study was analysed using the statistical package for social sciences (SPSS) version 20.0 for windows (SPSS Institute, Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to compare means, followed by the Tukey's test correction.

Values were considered significant at $p \leq 0.05$. Post hoc multiple comparisons and descriptives for the differences between groups were established by least significance differences (LSD). All the data are expressed as Mean ± Standard Error of the Mean (SEM).

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Results

As shown in **Table 2,** at the 4th and 8th weeks of treatment, the serum urea levels were significantly higher ($p \leq 0.05$) in the diabetic control group (102.37 ± 6.48, 86.27 ± 20.84 and 76.38 ± 0.38) compared to the normal control group (55.44 ± 0.67, 60.17 ± 2.65 and 55.31 ± 0.31) as well as the diabetic groupss treated with actovista (64.51 ± 2.33, 33.55 ± 6.42 and 50.34 ± 0.34), antioxidant supplement (68.28 ± 3.14, 62.81 ± 3.81 and 26.57 ± 4.88) and RiboCeine® (74.09 ± 3.03, 37.11 ± 2.86 and 27.20 ± 3.30) respectively. Serum urea levels were significantly lower ($p \leq 0.05$) in the group administered actovista compared to those that received the antioxidant supplements after the 4th week.

Table 2 Serum urea level in normal and HFD-STZ induced NIDDM in albino rats administered antioxidant supplement.

Urea	Week 4	Week 8	Week 12
GP 1	55.44 ± 0.67	60.17 ± 2.65	55.31 ± 0.31
GP 2	102.37 ± 6.48[a]	86.27 ± 20.84[a]	76.38 ± 0.38[a]
GP 3	64.51 ± 2.33	33.55 ± 6.42[b]	50.34 ± 0.34
GP 4	68.28 ± 3.14[a]	62.81 ± 3.81	26.57 ± 4.88[b]
GP 5	74.09 ± 3.03[a]	37.11 ± 2.86[b]	27.20 ± 3.30[b]
Data represented as Mean ± SEM; [a] = significantly higher compared to the control group, [b] = significantly lower compared to the control group			

Serum creatinine levels as shown in **Table 3** was significantly higher ($p \leq 0.05$) in the diabetic control group (1.247 ± 0.38, 1.453 ± 0.01 and 1.622 ± 0.02) as against the normal control group (0.516 ± 0.03, 0.567 ± 0.03 and 0.448 ± 0.05) and the diabetic treated groups [Actovista (0.588 ± 0.04, 0.580 ± 0.00 and 0.411 ± 0.00), antioxidant supplement (0.606 ± 0.02, 0.603 ± 0.02 and 0.565 ± 0.01) and RiboCeine® (0.690 ± 0.02, 0.638 ± 0.05 and 0.549 ± 0.03)] respectively across the weeks (4, 8 and 12). However, there was no observed statistical difference ($p \geq 0.05$) in the creatinine levels between the normal control group as against the various treatment groups.

Table 3 Serum creatinine level in normal and HFD-STZ induced NIDDM in albino rats administered antioxidant supplement.

Creatinine	Week 4	Week 8	Week 12
GP 1	0.516 ± 0.03	0.567 ± 0.03	0.448 ± 0.05
GP 2	1.247 ± 0.38[a]	1.453 ± 0.01[a]	1.622 ± 0.02[a]
GP 3	0.588 ± 0.04	0.580 ± 0.00	0.411 ± 0.00
GP 4	0.606 ± 0.02[a]	0.603 ± 0.02[a]	0.565 ± 0.01[a]
GP 5	0.690 ± 0.02[a]	0.638 ± 0.05[a]	0.549 ± 0.03[a]
Data represented as Mean ± SEM; [a] = significantly higher compared to the control group, [b] = significantly lower compared to the control group.			

For the serum bicarbonate levels as shown in **Table 4,** there was no significant difference ($p \geq 0.05$) observed in the diabetic control group (33.04 ± 7.39) relative to the diabetic treated groups (27.40 ± 2.72, 26.12 ± 0.73 and 27.96 ± 0.30) at the 4th week. However, at the 8th and 12th week of treatment, the HFD/STZ-induced diabetic control group (34.64 ± 4.52 and 38.50 ± 0.50) had significantly higher ($p \leq 0.05$) serum bicarbonate levels compared to the normal control group (30.77 ± 0.60 and 27.33 ± 0.33), as well as the diabetic rats treated with Actovista (26.04 ± 2.40 and 30.64 ± 0.36), antioxidant supplement (28.08 ± 0.34 and 27.06 ± 1.38) and RiboCeine® (28.25 ± 1.88 and 31.50 ± 1.38) respectively. As observed, no significant difference ($p \geq 0.05$) was observed in the bicarbonate levels of the normal group as against the various treated groups.

Table 4 Serum biocarbonate level in normal and HFD-STZ induced NIDDM in albino rats administered antioxidant supplement.

Biocarbonate	Week 4	Week 8	Week 12
GP 1	31.51 ± 3.14	30.77 ± 0.60	27.33 ± 0.33
GP 2	33.04 ± 7.39[a]	34.64 ± 4.52[a]	38.50 ± 0.50[a]
GP 3	27.40 ± 2.72[b]	26.04 ± 2.40[b]	30.64 ± 0.36

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GP 4	26.12 ± 0.73[b]	28.08 ± 0.34[b]	27.06 ± 1.38
GP 5	27.96 ± 0.30	28.25 ± 1.88	31.50 ± 1.38[a]
Data represented as Mean ± SEM; [a] = significantly higher compared to the control group, [b] = significantly lower compared to the control group			

Table 5 revealed the electrolyte levels as at the 4th, 8th and 12th weeks of treatment. The serum potassium levels as observed from the diabetic control group (2.54 ± 0.24 and 2.66 ± 0.40) at the 4th and 8th week was significantly lower ($p \leq 0.05$) when compared to the normal control (4.23 ± 0.42 and 3.56 ± 0.42) and treated groups. However, at 12th week of study, the treated groups showed a significantly difference ($p \leq 0.05$) in serum potassium levels when compared across the various weeks.

Table 5 Serum potassium level in normal and HFD-STZ induced NIDDM in albino rats administered antioxidant supplement.

Potassium	Week 4	Week 8	Week 12
GP 1	4.23 ± 0.42	3.56 ± 0.42	3.31 ± 0.01
GP 2	2.54 ± 0.24[b]	2.66 ± 0.40[b]	2.11 ± 0.01[b]
GP 3	3.29 ± 0.03	3.25 ± 0.13	3.90 ± 0.01[a]
GP 4	3.29 ± 0.04[b]	3.33 ± 0.05	3.30 ± 0.41
GP 5	3.30 ± 0.04	3.37 ± 0.05	3.27 ± 0.17
Data represented as Mean ± SEM; [a] = significantly higher compared to the control group, [b] = significantly lower compared to the control group.			

The diabetic control group (100.90 ± 2.23, 117.40 ± 12.27 and 99.32 ± 0.32) had significantly lower ($p \leq 0.05$) serum sodium levels compared to the normal control group (120.38 ± 2.11, 159.88 ± 3.99 and 164.69 ± 0.31) as well as the diabetic treated groups (**Table 6**). There was no observed significant difference ($p \geq 0.05$) in the sodium levels of the diabetic treated groups (actovista, antioxidant supplement and RiboCeine®).

Table 6 Serum sodium level in normal and HFD-STZ induced NIDDM in albino rats administered antioxidant supplement.

Sodium	Week 4	Week 8	Week 12
GP 1	120.38 ± 2.11	159.88 ± 3.99	164.69 ± 0.31
GP 2	100.90 ± 2.23[b]	117.40 ± 12.27[b]	99.32 ± 0.32[b]
GP 3	139.51 ± 11.83[a]	139.07 ± 2.30[b]	139.44 ± 0.44[b]
GP 4	130.04 ± 7.64[a]	151.45 ± 1.28	158.32 ± 9.35
GP 5	140.20 ± 7.61[a]	142.36 ± 1.00	140.06 ± 1.57
Data represented as Mean ± SEM; [a] = significantly higher compared to the control group, [b] = significantly lower compared to the control group			

The serum chloride level as shown in **Table 7,** reveals that the diabetic control group had significantly lower ($p \leq 0.05$) levels compared to the diabetic treated groups. There was no observed significant difference ($p \geq 0.05$) in the chloride levels between the diabetic treated groups (actovista, antioxidant supplement and RiboCeine®) throughout the durations of study.

Table 7 Serum chloride level in normal and HFD-STZ induced NIDDM in albino rats administered antioxidant supplement.

Chloride	Week 4	Week 8	Week 12
GP 1	138.29 ± 1.12	208.18 ± 31.97	166.21 ± 0.21
GP 2	119.91 ± 1.43[b]	94.80 ± 1.96[b]	92.73 ± 0.27[b]
GP 3	163.20 ± 20.09[a]	148.33 ± 6.32	155.45 ± 0.45
GP 4	153.16 ± 5.58	132.90 ± 10.22[b]	158.43 ± 9.96

GP 5	155.23 ± 8.35	140.76 ± 3.21	139.10 ± 2.26
Data represented as Mean ± SEM; [a] = significantly higher compared to the control group, [b] = significantly lower compared to the control group.			

Discussion

Gluconeogensis is often times sustained by increased proteolysis which is associated with release of glucogenic amino acids that are deaminated in the liver, resulting in high urea levels [16]. A significant increase in serum urea and creatinine levels indicates an impaired renal function of diabetic animals [17]. The result showed a significant ($p<0.05$) decrease in serum urea and creatinine concentration of the treated groups when compared to those of the normal and diabetic control groups, and the decrease was consistent all through the treatment duration (**Tables 2 and 3**). The observed elevation in serum urea and creatinine levels in the diabetic rats when compared to normal control and treated groups is consistent with other studies [18] which revealed that diabetes may expedite renal malfunctioning which might not be unrelated to oxidative stress and the stimulation of gluconeogenesis, resulting from insulin deficiency. Administration of the antioxidant supplement and the standard drugs significantly ($p \leq 0.05$) decreased these markers in the treated groups when compared to the diabetic control group.

There was no observed statistically significant difference ($p \leq 0.05$) in biocarbonate and potassium levels of both the treated and untreated groups when compared with the results obtained from various weeks analysed. However, comparison between biocarbonate levels of the normal control and treated groups against the diabetic control group was significant ($p \leq 0.05$) lower, while that of potassium level was significantly ($p \leq 0.05$) higher.

One of the functions of the kidney is to maintain constant blood electrolyte concentrations despite physiologic body changes [19]. Hence, serum electrolyte values usually depicts renal functions or dysfunctions, since in cases of uncontrolled diabetes mellitus, kidney function is compromised. Glycosuria, which is a diagnostic feature of diabetes, causes dehydration via glucose osmotic dieuresis. Such dehydration is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates [20,21]. The result portrayed a decrease in serum electrolyte concentrations of diabetic control rats compared to the normal control and treated rats. This is consistent with reports of other researchers [19,21,22]. The antioxidant supplement and standard drug administration may have increased the serum electrolyte concentrations of the HFD-STZ induced diabetic rats, and this could be a useful tool in ameliorating the depletion in electrolyte levels under diabetic conditions.

These results show the ameliorative effects of the treatment regimen against diabetic-induced renal dysfunction in rats.

Conclusion

The study examined the effect of antioxidant supplementation and kidney function status of high fat diet and low dose streptozotocin induced type 2 diabetes mellitus in Wistar rats. The results therefore suggest that the supplement may be useful in ameliorating the effect of diabetes and oxidative stress related kidney dysfunction as well as the restoration of electrolyte levels resulting from diabetic conditions.

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