EFFECTS OF CO-ADMINISTRATION OF LYCOPENE AND/OR ZINC ON SERUM ELECTROLYTE IN ALLOXAN-INDUCED DIABETIC WISTAR RAT

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Abstract

Background: The incidence of diabetes is increasing rapidly with interference in electrolytes sodium (Na+), potassium (K+) and chloride (Cl−). This work was designed to study the effects of co-administration of Lycopene and/or Zinc on Serum Electrolyte in Alloxan-Induced Diabetic Wistar Rat.

Material and Methods: Healthy albino rats weighing between 150g and 200g were used. The rats were randomly allotted into six groups, each containing five albino rats respectively. Five of the groups (II, III IV V and VI) were induced with diabetes by single intraperitoneal (i.p) injection of freshly prepared in 0.1 mol/L citrate buffered solution (pH 4.5) of streptozotocin (Sigma Aldrich, St. Louis, MO, USA) at a dose of 150 mg/kg body weight. Control (vehicle) rats were injected with equal volume of 0.1 mol/L citrate buffer. Four days after Alloxan injection, diabetes induction was confirmed by measuring fasting blood glucose level in a tail vein blood samples using ACCU-CHEK compact plus glucometer (Roche, France). Rats with glucose level of 200 mg/dl or higher were considered as diabetic. After the induction of diabetes the rats were treated using the Lycopene and Zinc separately and in combination respectively according to group daily, whereas, the other group (I) was not given any treatment and this served as the normal control, providing a baseline data.

Results: The diabetic treated groups had significantly (P < 0.05) higher serum Na+ concentration than the diabetic control group. The diabetic treated groups had significantly (P < 0.05) lower serum K+ concentration than the diabetic control. Also, the diabetic treated groups had significantly (P < 0.05) higher Cl− concentration than the diabetic control group. The diabetic treated group had significantly (P < 0.05) lower HCO3− concentration than the diabetic control group. The diabetic treated groups had significantly (P < 0.05) lower serum urea concentration when compared to the diabetic control. The diabetic treated groups showed significantly (P < 0.05) decrease in serum creatinine concentration as compared to the diabetic control group.

Conclusion: These anomalies were all ameliorated to about normal values after four weeks of treatment with Lycopene+Zinc. This suggests the synergistic beneficial effects of lycopene acid and zinc against alloxan-induced diabetes in Wistar Rats.

Keywords

Diabetes mellitus, Alloxan, Lycopene, Zinc, Wistar Rats.

Introduction

Arrays of multisystem complications are known to be associated with both type I and II DM; including alterations in the function of the hepatobiliary and renal systems. The most common disease associated with DM in the biliary system, is the formation of gallstones. The pathogenesis of cholesterol gallstone formation is complex and many factors are involved, such as changes in bile composition (cholesterol, bile acids, and phospholipids), changes in gall bladder motility and the presence of nucleation promotion factors. Diabetics have abnormal serum lipid profiles and increased biliary cholesterol secretion, resulting in increased cholesterol saturation of bile1. Elevated plasma insulin has been associated with an increased prevalence of gallstone disease1 and may account for the strong association between, Type 2 Diabetes Mellitus and gall stones. The electrolytes in serum include sodium (Na+), potassium (K+), calcium (Ca2+) and magnesium (Mg2+)². These electrolytes play an important role in intermediary metabolism and cellular function, including enzyme activities and electrical gradients3. Serum concentrations of electrolytes have been shown to change with plasma glucose levels. Disturbances in the levels of some electrolytes are associated with diabetes mellitus (DM)4. In addition, hypomagnesemia and diuretic associated hypokalemia may lead to a higher incidence of DM5, mild electrolyte changes such as low Mg2+ levels can predict mortality in type 2 DM and oral magnesium supplementation reduces fasting plasma glucose levels in DM patients6. The Atherosclerosis Risk in Communities (ARIC) study has shown an association between low serum  

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magnesium level and an increased risk of ischemic stroke in African Americans and Caucasians.  

Materials And Methods

Experimental Animals

Thirty Wistar rats of both sexes weighing between 150 to 250g (aged six to eight weeks) were obtained and housed in the animal house unit of the Department of Human Physiology, Ahmadu Bello University, Zaria. The normal standard rat chow and tap water were provided ad libitum during the experiment. Animals were stabilized to acclimatize to animal house environment for one week before commencement of the experiment. The study protocol was approved by the Institutional Animal Ethics Committee of the University, Ahmadu Bello University, Zaria.

Methodology

Induction of Diabetes Mellitus

Diabetes was chemically induced by intraperitoneal (i.p) injection of freshly prepared in 0.1 mol/L citrate buffered solution (pH 4.5) of Alloxan (Sigma Aldrich, St. Louis, MO, USA) at a dose of 150 mg/kg body weight. Control (vehicle) rats were injected with equal volume of 0.1 mol/L citrate buffer. Four days after Alloxan injection, diabetes induction was confirmed by measuring fasting blood glucose level in a tail vein blood samples using ACCU-CHEK compact plus glucometer (Roche, France). Glucose levels of diabetic rats were aspirated for at least 5 minutes to clean the system.

Kidney Function Test

Sodium (Na⁺) and potassium (K⁺) were measured by the flame photometry method of 10.

Procedure: The instrument was allowed to warm up for 5-10 minutes. Distilled water was fed to the instrument. The element Na⁺ was selected by turning the selector “Elementwahl”. The outer knob “Messbereich” was turned into position “100”. The “Kompensation I” knob was pulled slightly out and adjusts readout to 0. The “Kompensation I” knob was pressed back. The reading 0 was readjusted with “Kompensation II”. The most concentrated standard solution was aspirated and adjusted readout to approximately 350 (on uppermost scale) using inner “Messbereich” knob. Distilled water was aspirated-the instrument should read 0. Standard solutions no. 1, 2, 3, test solution were aspirated, and then standards 4, 5, 6. results were then recorded. The procedure was repeated 3-7 for solutions of potassium. Distilled water was aspirated for at least 5 minutes to clean the system.

Chloride

Chloride (Cl⁻) ion was measured using the method of 11. 

Procedure: This entire procedure was best carried out directly in the spectrophotometer tubes.

0.5 mL of serum was transfer to each of two spectrophotometer tubes. To one tube 15.0 mL of colour reagent was added; to the second tube add 15.0 mL of reagent blank. The tubes were shaken as the reagents were added. A slight precipitation was form, but this disappeared on standing. Ten minutes was allowed for the color to develop and also for the precipitate to dissolve. The spectrophotometer was set at zero absorbance at 480 m with the blank and then obtains the absorbance of the unknown. The concentration of the unknown was obtained from the precalibrated card or standard curve, whichever is preferred.

Bicarbonate

Bicarbonate (HCO₃⁻) was determined using the flame photometry method of 12. Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of PEPCase to produce oxaloacetate and phosphate. This reaction occurs in conjunction with the transfer of a hydrogen ion from NADH to oxaloacetate using malate dehydrogenase. The resultant formation of NAD caused a decrease in absorbance in the UV range (320–400 nm). The change in absorbance was directly proportional to the concentration of bicarbonate in the sample being assayed.

Procedure: After clot formation, blood samples were centrifuged immediately to obtain clear unhaemolized serum. 0.1 mL of serum was pipetted into the specially designed centrifuge tubes and stoppered. During the remainder of the procedure, contact of the solution with the atmospheric air is kept to a minimum by rapid replacement of the stopper after each manipulation. 0.9 mL of distilled water from a Machlett burette was added, and the solution in the tube is mixed by gentle tapping. At the same time, a blank was prepared containing 1 mL of the distilled water. From this point, both the blank and the sample are treated identically. Saturated Ba(OH)₂ reagent 0.3 mL was now added, and the solution was again mixed gently. After less than 1 minute, precipitation takes place; 0.5 mL of ethanol-water wash solution, was added, and the solution is mixed again. After centrifuging the samples for 10 minutes at 2000 r.p.m., the supernatant fluid was aspirated slowly by means of the Nz aspiration apparatus. This was accomplished without disturbing the precipitate at the bottom or touching the sides of the tube with the capillary. The precipitate was

Blood Collection

At the end of the experimental period of four weeks, overnight fasted animals were anaeathesized by halothane. About 5 mL of blood was aseptically collected by cardiac puncture from each rat using a 5 mL syringe. The blood sample was placed in a bottle without anticoagulant for serum sample for biochemical analyses.

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re-suspended in the small volume of fluid remaining and washed first with 1 mL of the ethanol-water wash solution reagent and finally with 1 mL of freshly boiled deionized water. After each washing, the tubes were centrifuged, and the supernatant fluid was aspirated over nitrogen. At this point, the rubber stoppers were removed, and the tubes were dried at 110°C for approximately 20 minutes. After cooling, 0.5 mL of 2 M HCl was added to each tube and placed in a boiling water bath for 1 minute. The tubes were diluted to 5 mL with distilled water, stoppered, mixed, and then centrifuged. The samples were now ready for flame photometry. With the phototube shutter open, with water as a blank, the zero reading was adjusted with the dark current knob. Other adjustments comprise hydrogen gas pressure at the control panel, 4.5 pounds per sq. in.; oxygen gas pressure, 15 pounds per sq. in.; monochrometer sensitivity, maximum counter-clockwise; slit width, approximately 0.1 mm; wave-length, 493.4 rnp; photomultiplier sensitivity, 4; zero suppression. By means of the working standard BaCl2 solution, the percent transmission was set to 50 with the slit adjustment. As a result, no calculation of volume percent CO2 was necessary. Percent transmission equals volume percent CO2. With water as the blank, each sample was then analyzed by flame photometry.

**Blood Urea Nitrogen (BUN)** Urea was determined by the method described by12. It was hydrolyzed by urease to form CO2 and ammonia. The ammonia formed then reacted with α-ketoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NAD+. The decrease in absorbance due to consumption of NADH was measured.

**Procedure:** Four vials were marked, “Blank,” “Standard 30,” “Standard 60,” and “Unknown,” and 5.0 mL of urea reagent was added to each vial. Since the final colour is developed in these vials, they may be cuvets. 20 µL of the urea was transferred to standards and 20 µL of serum to the respective vials and mixed by inversion. 0.5 mL of the diacetyl monoxime reagent was added to all vials, and then mixed. All vials were removed 12 minutes later and immersed in cool tap water for 5 minutes, and then mixed by inversion. The photometer was set on zero with the blank and read the absorbance of standards and unknown at 540 nm. A point-to-point standard curve was drawn. The absorbance of the color developed was not linear with concentration. If a standard curve is not used, the results will not be more than about 1 mg/100 mL low when calculations are made with the 30 mg/100 mL standard, for serum urea nitrogen concentrations up to 30 mg/100 mL. If the serum values are greater than 30 mg/100 mL, the sample should be reanalyzed with use of 10 ML. For samples suspected of having abnormally high urea values, a IO-czl sample should be used initially. This approach provides convenient one-point standardization for concentrations as great as 60 mg urea per 100 mL.

**Creatinine** Creatinine was determined by the method described by13. In an alkaline medium, creatinine forms a yellow-orange-colored complex with picric acid. The rate of colour formation was proportional to the concentration of creatinine present and which was measured photometrically.

**Procedure:** To the standard wells, 5 µL of creatinine standard was added (tubes A-H) per well in the designated wells on the plate. To the sample wells, 15 µL of each sample was added to at least two wells. 100 µL of creatinine reaction buffer was added to all the wells being used. 100 µL of creatinine colour reagent was added to all wells being used and immediately timing of the reaction was commenced. The absorbance was read and recorded 490-500 nm after one minute. At seven minute, the absorbance was read and recorded. Creatinine was then calculated as follow: Creatinine (mMol/L) = adjusted sample optical density - y-intercept) x sample dilution

**Slope**

**Statistical Analysis**

All data were expressed as Mean±SEM and data were analyzed using statistical package SPSS (version 20) followed by one way analysis of variance (ANOVA) with multiple comparisons. The Tukey’s post-hoc test was used to determine difference between groups. Values of p<0.05 was considered as statistically significant.

**Results**

**Effects of Treatment on Serum Electrolytes**

**Serum sodium ion concentration in the various experimental groups of rats**

The mean Na+ (mMol/L) concentrations were 146.00 ± 3.25 and 109.00 ± 2.24 in the normal control and diabetic control groups, respectively. The diabetic treated group (DB+Lyc: 142.60 ± 2.65; DB+Zn: 122.97 ± 5.27 and DB+Lyc+Zn: 144.77 ± 3.18, respectively) had significantly (P < 0.05) higher serum Na+ concentration than the diabetic control group (Table 1).

**Serum potassium ion concentration in the various experimental groups of rats**

The mean K+ (mMol/L) concentrations were 6.35 ± 0.13 and 7.50 ± 0.23 in the normal and diabetic control groups, respectively (Table 4.8). The diabetic treated groups (DB+Lyc: 6.59 ± 0.52; DB+Zn: 5.23 ± 0.12 and DB+Lyc+Zn: 5.39 ± 0.17, respectively) had significantly (P < 0.05) lower serum K+ concentration than the diabetic control group (Table 1).

**Serum chloride ion concentration in the various experimental groups of rat**

The mean Cl- (mMol/L) concentrations were 98.20 ± 3.35 and 76.80 ± 1.56 in the normal control and diabetic control groups, respectively. The diabetic treated group (DB+Lyc: 87.60 ± 2.32, DB+Zn: 101.00 ± 4.43 and DB+Lyc+Zn: 87.40 ± 2.11 respectively) had significantly (P < 0.05) higher Cl- concentration than the diabetic control group (Table 1).

DB: Diabetic, OLV: Olive oil, LYC: Lycopene, Zn: Zinc, GLIB: Glibenclamide, Mean ± SEM, n = 5. Values with error bars having different superscript letters are significant P<0.05 when compared with the diabetic control.
The present study evaluated the effect of co-administration of lycopene and/or zinc on serum electrolytes in alloxan-induced diabetic Wistar rat. Oral administration of lycopene alone and the combination of lycopene and zinc restored the serum sodium concentration to almost normal. It was found that there was a significant change in serum sodium ion concentration in groups treated with zinc alone and DB+Lyc+Zn when compared to the diabetic control group. The study also shows a significant increase in serum potassium ion concentration almost close to normal. It was found that there was significant P < 0.05 increase in serum potassium ion concentration when compared to the diabetic control group.

### Table 1. Effects of Co-administration of Lycopene and/or Zinc on Serum Electrolytes in Alloxan-Induced Diabetic Wistar Rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental Condition</th>
<th>Na+ (mMol/L)</th>
<th>K+ (mMol/L)</th>
<th>Cl− (mMol/L)</th>
<th>HCO3− (mMol/L)</th>
<th>Urea (mMol/L)</th>
<th>Creatinine (mMol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>146.63 ± 0.35</td>
<td>6.35 ± 0.13</td>
<td>98.80 ± 3.35</td>
<td>48.00 ± 1.54</td>
<td>34.28 ± 0.94</td>
<td>0.94 ± 0.05</td>
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<tr>
<td>2</td>
<td>DB+OLV</td>
<td>109.58 ± 2.42</td>
<td>7.50 ± 0.23</td>
<td>76.8 ± 1.98</td>
<td>34.00 ± 1.41</td>
<td>55.00 ± 1.58</td>
<td>1.60 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>DB+LYC</td>
<td>142.58 ± 2.65</td>
<td>6.59 ± 0.52</td>
<td>87.6 ± 3.32</td>
<td>22.80 ± 1.56</td>
<td>44.39 ± 2.62</td>
<td>1.03 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>DB+Zn</td>
<td>122.97 ± 3.14</td>
<td>5.23 ± 0.52</td>
<td>101.0 ± 2.23</td>
<td>19.00 ± 0.32</td>
<td>41.10 ± 1.63</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>DB+LYC + Zn</td>
<td>144.77 ± 3.81</td>
<td>5.39 ± 0.17</td>
<td>87.40 ± 2.11</td>
<td>19.6 ± 0.81</td>
<td>37.86 ± 1.78</td>
<td>0.18 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>DB+GLIB</td>
<td>121.20 ± 1.63</td>
<td>5.10 ± 0.17</td>
<td>83.3 ± 1.56</td>
<td>28.00 ± 1.41</td>
<td>40.00 ± 1.58</td>
<td>1.60 ± 0.02</td>
</tr>
</tbody>
</table>

**Discussion**

The present study evaluated the effect of co-administration of lycopene and zinc supplements on serum electrolytes in alloxan-induced diabetes mellitus. Results obtained shows altered electrolyte levels in diabetic rats. The study showed a significant increase in serum urea and creatinine levels in diabetic control rats in comparison with normal control rats. Plasma urea are recognized makers of glomerular filtration and in nephropathy. This result is in agreement with the report of who showed increased serum urea level in diabetic patients. An elevation of serum urea usually signifies decreased renal function. In diabetes, there is increased catabolism of amino acids resulting in high urea formation from the urea cycle and reduced filtering capacity of the kidney which leads to accumulation of waste products within the system of diabetic animals. A research conducted by had found that increase urea and serum creatinine in diabetic rats indicate progressive renal damage. Treatment of diabetic rats with the supplements produced a significant reduced serum urea, suggesting their ability to protect against diabetes-induced kidney damage, by preventing altered protein metabolism and/or impaired renal function that often exist in diabetes mellitus. Diabetes is characterized by increased volume and metabolites excretion via the kidney, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes.

The present study showed significant decrease in serum potassium ion concentration in groups treated with zinc alone and DB+Lyc+Zn when compared to the diabetic control group. Oral administration of lycopene alone and the combination of lycopene and zinc restored the serum sodium ion concentration in all the treated groups when compared to the diabetic control group. The study also shows a significant increase in serum potassium ion concentration in all the treated groups when compared to the diabetic control group. The result was found to be in agreement with that of who reported high sodium ion level in hyperosmolarity of DM.
Conclusion

These anomalies were all ameliorated to about normal values after four weeks of treatment with LYC+Zn. This suggests the synergistic beneficial effects of Lycopene and Zinc against Alloxan-induced diabetes in Wistar Rats.

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References