Modulatory roles of vitamin C and E on blood glucose and serum electrolytes levels in fructose-induced insulin resistance (Type 2) diabetes mellitus in wistar rats

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ABSTRACT

Diabetes mellitus is a disease that affects not only glucose metabolism but also mineral metabolism. This study was conducted to evaluate the effects of vitamin C and E on blood glucose and electrolyte levels in fructose-induced hyperglycemia in Wistar rats. Twenty (24) Wistar rats were used for the study. Each animal, regardless of their weight was made diabetic by feeding them with 20% (20g/100ml) of fructose dissolved in distilled water for a period of six (6) weeks, after which they were randomly assigned into four groups of six (6) animals each as follows: Group 1 served as diabetic control, Group 2 and 3 were treated with 100mg/kg b w of vitamin C and vitamin E respectively while Group 4 were treated with 250mg/kg b w of metformin and served as a positive control. All doses were administered orally once daily for a period of seven days. The results showed a statistically significant reduction (p<0.05) on blood glucose level in the groups treated with 100mg/kg b w of Vitamin C and E after day 3 and 7 when compared to control group. The result obtained also demonstrated a significantly reduced (p<0.05) serum sodium ion level in all groups treated with 100 mg/kg b w of vitamin C and E respectively when compared to diabetic control group. In regards to serum potassium ion, only Vitamin C at tested dose of 100mg/kg b w produced a significant change when compared to diabetic control group. However, the serum bicarbonate level was significantly decrease (p<0.05) in all groups treated with Vitamin C (100 mg/kg b w) and Vitamin E (100 mg/kg b w) respectively when compared to control group non treated group.

Key words: Diabetes mellitus, vitamin C, vitamin E, fructose, serum electrolytes, metformin

INTRODUCTION

Diabetes mellitus is a disorder in which the body is unable to metabolize carbohydrates properly. The disease is characterized by excessive amounts of sugar in the blood and urine; inadequate production and/or utilization of insulin; and by thirst, hunger and loss of weight [1]. Type 2 diabetes mellitus accounts for approximately 85 % of all cases of diabetes mellitus and is an important risk factor for cardiovascular morbidity and mortality[2]. Fructose, a natural sugar found in many fruits, is consumed in significant amounts in Western diets [3]. Electrolyte imbalance secondary to compromise in kidney function in prolonged and uncontrolled hyperglycemia of diabetes mellitus has long been established.Usually, glycosuria, a prominent diagnostic feature of diabetes mellitus imposes dehydration via glucose osmotic diuresis, which is usually accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates [4]. Ingestion of high doses of fructose over a prolonged period has been used to induce persistent hyperglycaemia rats with features similar to those seen in patients with type 2
diabetes mellitus (DM), hence its use in type 2-like DM induction in animals[5]. Vitamin C is a water-soluble vitamin that is found intra- and extracellularly as ascorbate [6]. It is a natural antioxidant that prevents the increased production of FRs induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues[7]. It has been shown to react directly with superoxide[8], hydroxyl radicals [9] and singlet oxygen [10]. It is generally regarded as a primary first-line protective agent that repairs or nullifies FRs by donating a single electron, followed by a proton to yield a chemically reduced non-radical product and ascorbyl radical. The ascorbyl radical dismutates to ascorbate and dehydroascorbic acid[11]. Vitamin E, the principal lipid-soluble antioxidant [12]. Vitamin E includes 8 naturally occurring forms, which can be divided into two families of compounds, the tocopherols and the tocotrienols; collectively known as tocols [13]. Specifically, vitamin E is able to extinguish single oxygen species as well as to terminate free radical chain reactions [14]. Alpha-tocopherol acts as an antioxidant either by donating a hydrogen radical to remove the free lipid radical, reacting with it to form non-radical products, or simply trapping the lipid radical [15]. This protective activity of vitamin E depends on vitamin C to recycle oxidized vitamin E [16]. The present study is aim at evaluating the effect of Vitamin C and E on blood glucose levels and serum electrolyte levels on fructose-induced insulin resistance (Type 2) diabetes mellitus on Wistar rats.

MATERIALS AND METHODS

Chemicals used
All chemicals and drugs used were of analytical grade. Alloxan was purchased from (Sigma chemical Company St. Louis U.S.A.). A digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) was used for the determination of the blood glucose levels of the animals.

Drugs used
Vitamin C and E were obtained from was obtained from a pharmaceutical store in Zaria. Each tablet of ascorbic acid (100 mg; Med Vit C®, Dol-Med Laboratories Limited, Lagos, Nigeria was reconstituted to 100 mg/mL suspension, just prior to its daily administration while Vitamin E (100 mg/capsule) each capsule was aspirated into a syringe and then reconstituted with soya oil to 100% v/v prior to daily administration.

Animals
Twenty (24) healthy Wistar rats of both sexes between the ages of 10-12 weeks old, weighed between 150-200 g were used for the study. The animals were kept in well aerated laboratory cages in the Department of Human Physiology and were allowed to acclimatize to the laboratory environment for a period of one week before the study commenced. They were maintained on standard animal feeds and drinking water ad libitum. The Principle of laboratory animal care “(NIH publication No 85-23)” guideline and procedures were followed in this study (NIH publication reserved 1985).

Experimental induction of diabetes mellitus
D-Fructose (BDH, Poole, England) with a molecular weight of 180.16 was used for the study. Each rat, regardless of their weight was made diabetic by feeding them with 20% (20g/100ml) of fructose dissolved in distilled water for a period of six (6) weeks [17].

Group 1: Diabetic control and received 1ml of distilled water
Group 2: Received 100mg/kg b w of Vitamin E
Group 3: Received 100mg/kg b w of Vitamin C
Group 4: Received 250mg/kg b w of Metformin
The administration was by oral gavage spanning for a period of two weeks.

Determination of blood glucose level
All blood samples were collected from the tail vein of the rats at intervals of 0, 3, 5 and 7 days. Fasting blood glucose levels were determined by using glucose oxidase method [18] using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) and the results were expressed in the unit of mg/dl [19]. Rats having fasting blood glucose level ≥ 130 mg/dl were considered as diabetic.

Collection and preparation samples
After the last day of administration the animals were euthanized and blood samples were drawn from the heart of each by cardiac puncture into plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 15 minutes and the serum collected and then subjected to biochemical assays.

Estimation serum electrolytes
Serum sodium and potassium ions were measured by the flame photometry method of Vogel (1960) and bicarbonate ion was determined using the titration method of[20], Chloride ion was analysed using the method of [21].

Statistical analysis
Data collected from the control and experimental animals were expressed as mean ± SEM. The data were statistically analyzed using ANOVA with multiply comparisons versus control group. The values of p < 0.05 were considered significant[22].

RESULTS

Effect of Vitamin C and E on blood glucose level of fructose-induced diabetic wistar rats
Day 0 mean the blood glucose level of fructose-induced diabetic before the commencement of treatment. The results showed a statistically significant reduction (p<0.05) on blood glucose level in the groups administered with Vitamin C (100mg/kg bw) and E (100mg/kg bw) after day 3 and day 7 when compared to control group. However, the observed decrease on the level of blood glucose in these groups is comparable to metformin (250mg/kg bw) (Table 1).

Effect of Vitamin C and E on serum electrolytes in fructose-induced diabetic wistar rats
Result obtained in this study revealed a significantly reduction (p<0.05) on serum sodium ion in all groups treated with Vitamin C and E when compared to control group. In regards to serum potassium ion, only Vitamin C at tested dose of 100mg/kg bw produced a significant change when compared to diabetic control group. There was no significant change (p>0.05) on serum chloride ion in the groups treated with Vitamin C (100mg/kg bw) and E (100 mg/kg bw) when compared to control group. However, the serum bio-carbonate level was significantly depleted (p<0.05) in all groups treated with Vitamin C (100 mg/kg bw) and E (100 mg/kg bw) respectively when compared to control group (Table 2).

<table>
<thead>
<tr>
<th>Treatment given</th>
<th>Fasting blood glucose level (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>131±3.66</td>
</tr>
<tr>
<td>Vitamin C (100mg/kg bw)</td>
<td>138.2±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E (100mg/kg bw)</td>
<td>134.8±2.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metformin (250mg/kg bw)</td>
<td>136.8±2.61&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

Values presented as mean ± SEM
<sup>a</sup>p < 0.05 is statistically significant when compared to control group while ns= non significant.

<table>
<thead>
<tr>
<th>Treatment Given</th>
<th>Na&lt;sup&gt;-&lt;/sup&gt; (mmol/L)</th>
<th>K&lt;sup&gt;+&lt;/sup&gt; (mmol/L)</th>
<th>Cl&lt;sup&gt;-&lt;/sup&gt; (mmol/L)</th>
<th>HCO&lt;sub&gt;3&lt;/sub&gt;- (mmol/L)</th>
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</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>150.4±7.80</td>
<td>4.20±0.14</td>
<td>98.4±1.43</td>
<td>30.0±1.86</td>
</tr>
<tr>
<td>Vitamin C (100mg/kg bw)</td>
<td>107.0±4.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.4±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.2±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E (100mg/kg bw)</td>
<td>113.0±6.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.86±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.8±1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.2±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metformin (250mg/kg bw)</td>
<td>90.6±3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.44±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.0±2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values presented as mean ± SEM
<sup>a</sup>p < 0.05 is statistically significant when compared to control group while ns= non significant.

DISCUSSION

Ingestion of high doses of fructose over a prolonged period has been used to induce persistent hyperglycaemia in rats with features similar to those seen in patients with type 2 diabetes mellitus (DM), hence its use in type 2 diabetes mellitus induction in animals [5 ]. The method of [17] used in this study is acceptable in that there was a significant hyperglycemia following chronic ingestion of fructose. In this present study, feeding the animals with
high doses of fructose in the laboratory for a period of six weeks resulted in progressive significant increase in blood glucose level. In this study, administration of 100mg/kg bw of vitamin C resulted to significant decrease in blood glucose level in the diabetic rats when compared to the diabetic control group. Vitamin C may play an important role in physiological reactions such as mixed function oxidation involving incorporation of oxygen into a biochemical substrate. In addition, vitamin C is considered the most important antioxidant in extracellular fluids and its antioxidant function has been shown to efficiently scavenge superoxide, hydrogen peroxide, hydroxyl, peroxyl and singlet oxygen radicals[23;24]. Therefore, the antioxidant function of vitamin C as observed in this work is related to its reversible oxidation and reduction characteristics [23]. Vitamin C has been reported to efficiently scavenge free radicals before they can initiate lipid peroxidation, and contribute to stability of cellular and basal membranes [7]. Similarly, treatment of the diabetic animals with 100mg/kg bw of vitamin E significantly reduced the blood glucose level when compared with the control group. This essential fat-soluble vitamin functions primarily as an antioxidant[25]. Our findings in this present work is in agreement with the report of [26,27] who demonstrated that there was positive effects on diabetes mellitus and improvements in glycemic control from vitamin E supplementation. The hypoglycemic effects of both vitamins are comparable to the standard drug metformin. Synergism between vitamins C and E have been demonstrated [28]. Although both vitamins serve as free radical scavengers in biological system, vitamin C is hydrophilic and exerts its effect in the extracellular space, trapping radicals in the aqueous phase [29], while vitamin E is a lipid soluble antioxidant within the cells, where the reactive metabolites are actually produced [28]. Furthermore, vitamin C interacts with tocopheroxyl radical and generates the reduced tocopherol[30]. Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, and blood clotting and muscle contraction. Electrolyte imbalance resulting from kidney failure, dehydration, and fever and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders [31]. Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes [32]. In the present study treatment of fructose-induced diabetic animals with vitamin C (100mg/kg bw) and E (100 mg/kg bw) produced a significant decrease on serum sodium ion concentration; however vitamin E (100 mg/kg bw) did not produce a significant change on the level and potassium ion concentration. On other hand, administration of vitamin C (100 mg/kg bw) and E (100 mg/kg bw) recorded a non significant difference on serum chloride ion level in the diabetic animals when compared to diabetic untreated rats.

CONCLUSION

The results obtained in our study demonstrated that vitamin C and E at tested doses significantly reduced blood glucose level. However, there was a significantly decreased serum sodium ion and bio-carbonate in all groups that received Vitamin C and E. In regards to serum potassium ion, only Vitamin C at tested dose of 100mg/kg bw produced a significant change when compared to diabetic control group.

REFERENCES


