VIRGIN COCONUT OIL (VCO) SUPPLEMENTED DIET AMELIORATES BLOOD GLUCOSE, RENAL TISSUE AND ANTIOXIDANT ENZYMES IN DIABETIC RATS

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AUTHORS’ CONTRIBUTIONS
This work was carried out in collaboration between all authors. Author AMA designed the study, wrote the manuscript and interpreted the data. Authors SOJ and OB participated in experimental design and data interpretation. Authors IEO and TBO carried out the analysis of blood glucose and managed the literature searches. Authors TBO and VES carried out the analysis of renal tissue and antioxidant enzymes. All authors read and approved the final manuscript.

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ABSTRACT
Untreated hyperglycaemia leads to kidney damage through oxidative stress of the kidney tissues with resultant compromise of the antioxidant enzymes. According to research, Virgin Coconut Oil (VCO) has been reported to have hypoglycaemic and anti-diabetic properties. Therefore, this study was designed to investigate the ameliorative effects of VCO supplemented diet on blood glucose, renal tissues and antioxidant enzymes in diabetic rats. The study was carried out on fifteen male rats weighing 150 g-200 g and divided into 3 groups as follows: Normal control group (Group 1) and diabetic control group (Group 2) fed on normal rat chow, diabetic test group (Group 3) fed on 10% VCO diet. Diabetes mellitus was induced by intraperitoneal injection of alloxan (150 mg/kg). After 3 days of diabetes induction, blood glucose was assessed and subsequently on 7th, 10th, 14th, 21st and 28th days. The result showed a significant increase in blood glucose on the 3rd, 7th and 10th day in Group 2 and Group 3 compared to Group 1 as well as a significant decrease in Group 3 on 14th, 21st and 28th days. The SOD and CAT activities in kidneys significantly increased (p<0.05) and MDA in kidneys significantly reduced in Group 3 when compared to Group 2. Also GGT and ALP activities significantly increased in Group 3 when compared to Group 2. In conclusion, VCO can ameliorate hyperglycaemia, oxidative stress and enhances the activities of renal tissue enzymes in diabetics.

Keywords: Virgin coconut oil; diabetes mellitus; diabetic rats; diet; renal tissue enzyme; antioxidant enzymes; oxidative stress; oxidative stress biomarkers.

1. INTRODUCTION
Diabetes mellitus is a metabolic disease associated with kidney damage and sustained increase in blood glucose possibly due to lack of insulin secretion or insulin resistance to glucose metabolism as a result of oxidative stress. Its resultant effect on the general metabolic state of patients is evident in the kidney
tissues signaling to various indications (such as glucosuria (glucose in urine), polyuria (excessive urine), nocturia (excessive urination at night)) creating burden on kidneys. These indications associated with kidneys are suggesting that kidney tissues are overstrained with alterations in its tissue and antioxidant enzymes due to oxidative stress.

Apart from the metabolism of all the main food stuffs and antioxidant enzymatic actions that are altered, the physiologic capacity of kidney tissues is seriously reduced and embarrassed in untreated diabetic patients. Therefore, the absence of an effective cure for the diseases is a course for concern. Currently, several hundred plants have been reported to have beneficial effects in the treatment of diabetes [1,2]. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes mellitus and its secondary complications continue to be major medical problem to people.

However, dietary intake of different food substance such as virgin coconut oil has been reported as a measure of managing diabetes mellitus [3,4] and renal dysfunction [5] apart from the use of drugs. For instance, virgin coconut oil is consumed in tropical countries for thousands of years and studies done on native diets high in virgin coconut oil consumption showed that this population is generally good in health. Virgin coconut oil is antiviral, antifungal (kills yeast too) and antibacterial [6] In addition, the oil is rich in medium chain fatty acid (MCFA) and exhibit good digestibility [7], and various fractions of virgin coconut oil are used as drugs. Lauric acid is effective in treating viral infections [8], MCFA may help with weight maintenance in AIDS patients.

Therefore, virgin coconut oil has been exploited for it many health, dietary and nutritional benefits, but its effects on blood glucose level, renal tissue and antioxidant enzymes in alloxan induced diabetic rats are scarcely reported thus necessitating this research.

2. MATERIALS AND METHODS

2.1 Plants

2.1.1 Preparation of Virgin Coconut oil (VCO)

Dry coconuts were purchased from Okuku markets in the northern part of Cross River State, Nigeria. The VCO was extracted by wet extraction method [9]. The preparation of the 10% virgin coconut oil meal was done on regular demand.

2.2 Experimental Animals

Fifteen (15) male rats weighing 150-200 g were used in this study. The rats were purchased at the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH), Okuku Campus, Nigeria. The animals were kept in cages with suitable temperature, humidity, water and normal rat chow for 2 weeks to acclimatize. After 2 weeks of acclimatization, the animals were fasted for 12 hours and fasting blood glucose were assessed prior to the induction of diabetes mellitus.

2.3 Experimental Procedure

This study was carried out on 3 groups of rats which comprise of normal control group (Group 1), diabetic control group (Group 2) and diabetic test group (Group 3); each group consist of 5 rats and were placed in different cages for proper identification. However, all experiments on the animals were carried out in absolute compliance with ethical guideline for research, care and use of laboratory animals.

2.3.1 Group I-normal control group

The animals in this group served as control and were fed a normal rat chow and water ad libitum for 4 weeks.

2.3.2 Group II-diabetic control group

The animals were injected intraperitoneally with 150 mg/kg\(^{-1}\) of alloxan to induce diabetes mellitus. The animals were also fed a normal rat chow and water ad libitum for 4 weeks after diabetes induction.

2.3.3 Group III-diabetic test group

Also, each animal in this group was injected intraperitoneally with 150 mg/kg\(^{-1}\) of alloxan. The animals were fed a 10% virgin coconut oil diet and water ad libitum for 4 weeks after diabetes induction.

2.4 Renal Tissue and Antioxidant Enzymes Analysis

After 28th day, all the animals were sacrificed by decapitation. Immediately after decapitation, the kidneys were removed from the animals, washed with ice cold saline and their wet weights were obtained using weighing balance. The kidneys were kept in refrigerator before analysis of renal tissue and antioxidant enzymes.

Activities of renal alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) were determined...
in renal homogenate via specific analytical kits (Randox, United Kingdom) using spectrophotometry method.

2.5 Determination of Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA) Activity

The levels of total SOD activity in the tissues were determined by the method of Misra and Fridovich [10]. Catalase activity was determined according to the method of Sinha [11]. The MDA concentration of the sample was calculated from the absorbance using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ according to the method of Adam-Vizi and Sergi [12].

2.6 Statistical Analysis

The results obtained were presented as the mean ± standard error of mean (SEM) and analyzed using analysis of variance (ANOVA) with post-hoc test (Least Significant Differences) through Statistics Package for the Social Sciences (SPSS) version 17. The results were considered significant at P<0.05.

3. RESULTS

3.1 Fasting Blood Glucose Analysis

3.1.1 Effects of VCO on blood glucose on the different days of treatment

As summarized in the Table 1, there was no significant difference in fasting blood glucose levels in the diabetic test group when compared with the diabetic control and normal control groups on the 1st day of treatment. However, there was significant increase in fasting blood glucose on the 3rd, 7th and 10th day in diabetes test group and diabetes control group when compared to control group.

As shown in Table 1, as the days progressed, the fasting blood glucose of diabetic control group on the 14th, 21st and 28th days significantly increased (P<0.05) when compared to that of the control group while the fasting blood glucose of diabetic test group significantly decreased (P<0.05) in all these days when compared to that of diabetic control group.

3.2 Renal Tissue Enzyme Analysis

3.2.1 Effect of VCO on GGT and ALP

In Table 2, There was significant difference (P<0.05) in GGT and ALP values of diabetic control group when compared to that of normal control and diabetic test group. On the other hand, there was no significant difference in GGT and ALP values of control group compared to diabetic test group.

3.3 Renal Antioxidant Enzymes Analysis

3.3.1 Effect of VCO on antioxidant enzymes

In Table 2, the MDA value of diabetic control group was significantly different (P<0.05) when compared to that of normal control and diabetic test groups. There was no significant difference in MDA value of normal control group compared to the diabetic test group (2.14±0.22 U/mg protein and 2.07±0.13 U/mg protein). The SOD (3.42±0.42 U/mg protein) and CAT (4.80±1.70 µ moles H$_2$O$_2$ consumed/ min/ mg protein) values of diabetic control group was significantly different (P<0.05) when compared to the normal control and diabetic test groups. Also, there was no significant difference in SOD and CAT values of control group compared to the diabetic test groups.

4. DISCUSSION

The present study examined the effect of VCO on blood glucose, renal tissue and antioxidant enzymes in alloxan induced diabetic rats. The onset of hyperglycemia occurs on 3rd, 7th, and 10th days in both diabetic groups while the control group was relatively normal. On the other hand, the diabetic test group began to respond to treatment with VCO on 14th, 21st and 28th days.

<p>| Table 1. Effects of VCO on blood glucose of control, diabetic control and diabetic test groups |
|-----------------------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Control group (mg/dl)</th>
<th>Diabetic control group (mg/dl)</th>
<th>Diabetes test group (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td></td>
<td>82.00±1.64</td>
<td>84.80±4.00</td>
<td>85.40±4.15</td>
</tr>
<tr>
<td>3rd day</td>
<td></td>
<td>77.80±5.00</td>
<td>334.80±6.48</td>
<td>339.40±3.15</td>
</tr>
<tr>
<td>7th day</td>
<td></td>
<td>88.60±5.97</td>
<td>346.20±7.47</td>
<td>304.60±9.55</td>
</tr>
<tr>
<td>10th day</td>
<td></td>
<td>80.40±5.68</td>
<td>352.40±5.85</td>
<td>308.00±2.80</td>
</tr>
<tr>
<td>14th day</td>
<td></td>
<td>73.20±6.94</td>
<td>354.80±4.59</td>
<td>158.40±2.25</td>
</tr>
<tr>
<td>21st day</td>
<td></td>
<td>98.60±1.56</td>
<td>357.40±8.60</td>
<td>143.20±2.13</td>
</tr>
<tr>
<td>28th day</td>
<td></td>
<td>77.00±2.58</td>
<td>383.00±10.50</td>
<td>146.00±2.25</td>
</tr>
</tbody>
</table>

*Significantly different from control group (p<0.05)
**Significantly different from diabetic control group (p<0.05)
Table 2. Effects of VCO on renal tissue and antioxidant enzymes of control, diabetic control and diabetic test groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Control group</th>
<th>Diabetic control group</th>
<th>Diabetic test group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GGT (IU/L)</td>
<td>280.70±17.78</td>
<td>180.88±19.24</td>
<td>300.85±20.80</td>
</tr>
<tr>
<td></td>
<td>ALP (IU/L)</td>
<td>1337.82±34.86</td>
<td>850.14±30.54</td>
<td>1393.98±32.81</td>
</tr>
<tr>
<td></td>
<td>MDA (mol/g tissue)</td>
<td>2.14±0.22</td>
<td>3.87±0.21</td>
<td>2.07±0.13</td>
</tr>
<tr>
<td></td>
<td>SOD (U/mg protein)</td>
<td>16.10±1.34</td>
<td>3.42±0.42</td>
<td>14.31±1.11</td>
</tr>
<tr>
<td></td>
<td>CAT (µ moles H₂O₂ consumed/min/mg protein)</td>
<td>44±4.70</td>
<td>4.8±1.70</td>
<td>40±3.00</td>
</tr>
</tbody>
</table>

Significantly different from Control group (p<0.05)  
Significantly different from Diabetic control group (p<0.05)

The virgin coconut oil (VCO) significantly reduced the fasting blood glucose level in diabetic test group at 14th, 21st and 28th days compared to diabetic control group, probably due to the antioxidant effect of VCO and especially phytochemical constituents of lauric acid and caprylic acid have been reported to have effects on glucose metabolism and improve insulin levels [13,14].

In addition, it has also been reported that lauric acid in virgin coconut oil has insulinitropic properties [15]. Therefore, the result of this study corresponds to the report of the previous researchers on hypoglycaemic properties of virgin coconut oil [15,16,17].

Moreover, findings from this study showed that dietary consumption of VCO in diabetic test group enhanced the activities of antioxidant enzymes compared to diabetic control group.

Interestingly, a study conducted has shown a similar result of VCO enhancing the activities of antioxidant enzymes and reduced lipid peroxidation in normal rats [9]. Also, it was reported that SOD and catalase activities significantly increased in diabetic treated rats with VCO compared to diabetic untreated rats while MDA activities significantly reduced in diabetic treated rats with VCO compared to diabetic untreated rats [14] thus corresponding to the result of this present study. According to this study, VCO has ameliorative effects on antioxidant enzymes in alloxan induced diabetic rats. This may probably be due to the active components (Lauric acid, Caprylic acid, Capric acid) of VCO that have been reported to have antioxidant activities.

Another important finding from this present study was that dietary consumption of VCO in alloxan induced diabetic rats also enhanced the activities of renal tissue enzymes such as GGT and ALP in diabetic rats fed with VCO diets compared to the activities of this enzymes in untreated diabetic rats. However, the overall effect of VCO was better in enhancing the antioxidant status and renal tissue enzymes activities.

5. CONCLUSION

In conclusion, this study showed that dietary consumption of VCO in alloxan induced diabetic rats enhances the activities of oxidative stress biomarkers such as MDA, SOD, CAT, and renal tissue enzymes such as GGT and ALP.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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