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Histoarchitectural and Behavioral Characterization of the Prefrontal Cortex of Type 2 Diabetes Mellitus Male Wistar Rat and Neuroprotective Role of Virgin Coconut Oil

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Authors’ contributions

This work was carried out in collaboration between all authors. Author JAO designed the study and wrote the protocol. Authors SYO and JOO wrote the first draft of the manuscript and performed the statistical analysis. Authors JAO, JOO and ABOD managed the analyses of the study. Authors UEU and JAO managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: This research is aimed at investigating the possible effects of Virgin Coconut Oil on Histomorphology of the prefrontal cortex of type 2 diabetes male Wister rat.

Study Design and Methodology: Animals used for this research experiment was 32 male Wistar rats, and were grouped as follows;

Group A- Control group received water and feed ad libitum.
Group B – Received 0.6ml of Virgin coconut oil for 56 days
Group C – Received High-fat diet and 35 mg/kg of streptozotocin for 3days
Group D – Received High-fat diet ad libitum for 56days, 35mg/kg of streptozotocin for 3 days and then 0.6ml virgin coconut oil for 28 days.

The body weights of the animals were measured every week using a weighing balance. The
neurobehavioral analysis was carried out using Barnes maze to measure spatial learning and memory. The animals were sacrificed by cervical dislocation at the end of the experiment and the tissue removed for histological procedures which include: Haematoxylin & Eosin, and Cresyl fast Violet.

**Place and Duration of Study:** This work was carried out at Department of Anatomy Ben Carson School of Medicine Babcock University Ilishan Remo, Ogun State Nigeria and the experiment started on the 1st of February and ended on the 14th of March, 2017.

**Results:** Diabetes mellitus group lost a significant amount of weight, But the Virgin Coconut oil group animals added a significant amount of weight which suggests that VCO helps improve appetite.

The diabetes mellitus group had the highest latency in the Barnes maze test while the Virgin Coconut Oil group had the lowest primary latency.

Normal histological features of the Control and VCO treated (control) groups did not show any observable altered panoramic morphological presentation of the PFC layers. VCO treated group showed slight degenerative changes (yellow arrows) and STZ group showed induced degenerative changes in the cortex and was characterized by fragmented pyramidal and granule cell layer with observable pyknotic cells.

**Conclusion:** Diabetes affected the prefrontal cortex negatively while virgin coconut oil seems to neutralize the adverse effect and tried to restore the integrity of the prefrontal cortex.

**Keywords:** Diabetes; virgin coconut oil (VCO); streptozotocin (STZ); prefrontal cortex.

1. INTRODUCTION

1.1 Background of Study

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world. Globally an estimated 422 million adults are living with Diabetes mellitus [1]. Diabetes at least doubles a person’s risk of early death, with equal rates in both male and females. In 2012 there were 1.5 million deaths worldwide directly caused by diabetes, It was the eighth leading cause of death among both sexes and the fifth leading cause of death in women in 2012 [1].

There are two main types of diabetes mellitus:

i. Type 1 diabetes, also called insulin dependent diabetes mellitus (IDDM), is caused by lack of insulin secretion by beta cells of the pancreas. Type 1 diabetes is the result of an autoimmune reaction to proteins of the islets cells of the pancreas [2].

ii. Type 2 diabetes, also called non-insulin dependent diabetes mellitus (NIDDM), is caused by decreased sensitivity of target tissues to insulin.

The reduced sensitivity to insulin is often called insulin resistance. In both types of diabetes mellitus, metabolism of all the main foodstuffs is altered. The basic effect of insulin deficiency or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain [3].

Type 2 Diabetes mellitus was first described as a component of metabolic syndrome in 1988 [4]. The majority of the Diabetes mellitus burden in Africa appears to be type 2 Diabetes Mellitus, with less than 10% of Diabetes Mellitus cases being type 1 Diabetes Mellitus. Type 2 Diabetes Mellitus (formerly known as non-insulin dependent Diabetes mellitus) is the most common form of Diabetes mellitus characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [5]. In Type 2 Diabetes mellitus, insulin is no longer able to reduce the levels of blood glucose after a meal. The reason for this in most cases is that the insulin messenger signal no longer triggers the cellular cascade of events that leads to an increased uptake of glucose by cells [6].

Type 2 diabetes appears to be related to aging, inactive lifestyle, genetic influence, and obesity. As a result of this trend, it is fast becoming an epidemic in some countries of the world. In the condition, the body usually still produces a smaller amount of insulin but persistently high intakes of dietary sugars lead to excess demand on insulin production and the body cells do not properly respond to insulin, which leads to insulin resistance over time. Type 2 Diabetes mellitus has been seen to have an effect on the cognitive process which is one of the major functions of
the prefrontal cortex. The present studies have confirmed that Type 2 Diabetes Mellitus is a robust risk factor for cognitive dysfunction. However, the precise mechanisms remain to be elucidated [7]. In Luchsinger and colleagues’ study [8], the results indicated that Diabetes mellitus is related to a relatively higher risk for all causes of Mild Cognitive Impairment.

Overtime type 2 diabetes mellitus could lead to long-term medical problems such as:

- Stroke
- Heart disease
- Dental problems
- Stroke
- Foot problems
- Nerve damage
- Kidney problems, etc.

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [9]. It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes [10]. In diabetes mellitus, main sources of oxidative stress are mitochondria. During oxidative metabolism in mitochondria, a component of the utilized oxygen is reduced to water, and the remaining oxygen is transformed to oxygen free radical which is an important ROS [11].

1.2 The Prefrontal Cortex

The Prefrontal cortex is the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas. The prefrontal cortex is also called the dorsolateral prefrontal cortex. It comprises several Brodmann areas (BAs) anterior to the primary motor and premotor cortex. The PFC is involved in higher-level cognitive processes grouped under the term of “executive functions” in humans, including mostly dorsolateral areas, like BA 9, 10, and 46 [12]. The prefrontal cortex can be seen as the executive center of the brain. The frontal lobe and the portion of it occupied solely by association cortex, the prefrontal cortex, are eternally popular areas to research in human brain evolution due to their functional attributes.

1.3 Type 2 Diabetes and the Prefrontal Cortex

Cognition is defined as “the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses” (Oxford dictionary). Cognition is known as one of the major functions of the prefrontal cortex.

Diabetes Mellitus is related to 40% higher rate of mild cognitive impairment [8]. This is especially true when diabetes starts before the age of 65, or when the disease is more than 10 years. Treatment with insulin and the presence of diabetes complications such as retinopathy are other risk factors [13,14]. Insulin and its signaling pathways not only regulate glucose and energy metabolism but also modulate learning and memory [15]. As cognition-related structures such as the prefrontal cortex have a high density of insulin receptors and can produce insulin locally, an obstacle in any of the insulin signaling pathways can give rise to cognitive dysfunction most of which relates to memory, attention and executive functions [16].

In a large cross-sectional study, Moran et al. [17] reported that patients with T2DM had lower total grey, white, and hippocampal volumes. Regions with loss of grey matter include the medial temporal, anterior cingulate, and medial frontal lobes. White matter loss (WML) was found in the frontal and temporal regions. These investigators determined that brain volume loss was associated with poor performance in cognitive testing in these patients with T2DM. Other studies have suggested that atrophy may be greater in the prefrontal region in patients with T2DM [18,19]. Patients with T2DM have also been shown to have increased WMLs brain atrophy and WMLs have been associated with cognitive dysfunction in some but not all studies [20].

1.4 Virgin Coconut Oil

Virgin coconut oil is obtained from the fresh, mature kernel of the coconut by mechanical or natural means, with or without the use of heat, without undergoing chemical refining, bleaching or deodorizing, and which does not lead to the alteration of the nature of the oil [21]. It may retain native bioactive compounds present in it [22]. VCO extracted in cold and hot conditions is known to be rich in polyphenols. It is suitable for consumption without the need for further processing. It was developed to meet the needs of the natural foods market that advocate minimal processing of products. In recent years, virgin coconut oil has gained popularity as a nutraceutical. Promoted as a dietary supplement designed to optimize health through improved nutrition, it is said to be of benefit for patients
with various ailments. Unlike coconut (copra) oil, it is endowed with the natural antioxidant. Flavonoids and other polyphenols may be responsible for the antioxidant properties of VCO.

1.5 Virgin Coconut Oil and Type 2 Diabetes Mellitus

In diabetic patients, antioxidants may play a vital role in improving insulin response to the loaded glucose and may reduce insulin resistance. Antioxidant enzymes are a critical part of cellular protection against reactive oxygen species and ultimately oxidative stress. Since, oxidative stress contributes significantly to the pathophysiology of diabetes [23], substances that suppress oxidative stress might be therapeutically beneficial. Studies have shown that exogenously administered antioxidants have protective effects on diabetes, thus providing insight into the relationship between free radicals and diabetes [23].

As our local and global population increases there is a proportionate increase in the number of individuals with diabetes also increases; although the prevention of Type 2 Diabetes mellitus (T2 Diabetes Mellitus) is promoted worldwide there is still an increase in the population of people with diabetes. Present studies have confirmed that T2 Diabetes Mellitus is a robust risk factor for cognitive dysfunction. However, the precise mechanisms remain unclear [7]. Interestingly, insulin has direct effects on brain activity and cognitive processes. The impairment of insulin activity in the brain leaves neurons more exposed to toxic influences which could lead to cognitive dysfunction. To date, there are no clear treatments to help improve cognitive decline in patients with T2 Diabetes Mellitus [24]. This research is to try and investigate if Virgin Coconut oil will help improve cognitive dysfunction in a diabetic condition.

2. MATERIALS AND METHODS

The model animals used for this research experiment was the juvenile male Wistar rat, 32 male Wistar rats were used for the sake of this experiment. The Wistar rats were purchased from Babcock university animal house. The rats were left to acclimatize in Babcock university animal house for a period of 2 weeks. This was done to enable the rats to adapt to the new environment.

2.1 High Fat Diet

The high-fat diet is compounded following the formula in the Table 1 below:

<table>
<thead>
<tr>
<th>S/No</th>
<th>Ingredients</th>
<th>High Fat Diet (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Corn Starch</td>
<td>276</td>
</tr>
<tr>
<td>2.</td>
<td>Soya Beans</td>
<td>180</td>
</tr>
<tr>
<td>3.</td>
<td>Rice Husk (cellulose)</td>
<td>40</td>
</tr>
<tr>
<td>4.</td>
<td>Sucrose</td>
<td>80</td>
</tr>
<tr>
<td>5.</td>
<td>Soya bean Oil</td>
<td>50</td>
</tr>
<tr>
<td>6.</td>
<td>Vitamin/mineral mix</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>Methionine</td>
<td>4</td>
</tr>
<tr>
<td>8.</td>
<td>Lard/Fat</td>
<td>330</td>
</tr>
<tr>
<td>9.</td>
<td>Total</td>
<td>1000g</td>
</tr>
</tbody>
</table>

2.2 Experimental Design

The research was designed thus (Table 2):

Group A – the control group was given only water and feed ad libitum.

Group B – this group was given 0.6ml of Virgin coconut oil using an oral cannula for 56days

Group C – this group was given High-fat diet ad libitum and 35mg/kg of streptozotocin for 56days to induce type 2 diabetes mellitus

Group D – this group was given High-fat diet ad libitum for 56days, 35mg/kg of streptozotocin for 3 days from the 28th day to day 31, and then 0.6ml virgin coconut oil for 28 days to induce damage and then treatment.

During the course of this experiment, the rats were weighed weekly to monitor their weights and any possible changes that could occur.

2.3 Streptozotocin

Streptozotocin was obtained in the solid state. 0.38 g of Citrate was mixed in 20 ml of distilled water in a sample bottle. 0.516 g of sodium citrate salt was mixed in 20 ml of distilled water in a sample bottle.

5 ml of both was used as a buffer for the streptozotocin mixture.
Table 2. Table showing treatment regimen design for control and experimental groups

<table>
<thead>
<tr>
<th>S/N</th>
<th>Group</th>
<th>Treatments</th>
<th>Dosages [mg/kg]</th>
<th>Rationale</th>
<th>Duration [days]</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control [CO]</td>
<td>Water</td>
<td>Ad libitum</td>
<td>Placebo</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>Experimental B [VCO]</td>
<td>Virgin coconut oil</td>
<td>1700</td>
<td>Positive Control</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>3.</td>
<td>Experimental C [HFD+STZ]</td>
<td>High fat diet + streptozotocin</td>
<td>Ad libitum+ 35</td>
<td>Negative Control</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>4.</td>
<td>Experimental D [HFD+STZ+VCO]</td>
<td>High-fat diet then virgin coconut oil</td>
<td>1700</td>
<td>Therapeutic effect to induce damage then treatment</td>
<td>56</td>
<td>8</td>
</tr>
</tbody>
</table>

2.4 Virgin Coconut Oil Preparation

- The solid endosperm of matured coconuts was procured at the fruit grocery and authenticated as *Cocos nucifera* was done at the plant biology department of the University of Ilorin.
- The VCO was extracted using a modified wet extraction method described by Nevin and Rajamohan [25,26].
- The solid endosperm of mature coconut was crushed and made into a aluminium chlorides slurry.
- About 500 ml of water was added to the slurry obtained and squeezed through a fine sieve to obtain coconut milk.
- Demulsification produced layers of an aqueous phase (water) on the bottom, an emulsion phase (cream) in the middle layer and an oil phase on top as described by [28].
- The oil on top was aluminum chloride opened and heated for about 5 minutes to remove moisture.

The obtained VCO was filtered through a fine sieve, stored at room temperature and used for the experiment.

2.5 Measurements

The body weights of the animals were measured at the end of every week throughout the period of administration using a weighing balance. Glucose test was also conducted every Friday using a glucometer, small blood from the tip of the rat’s tail and a test strip to ascertain the changes in the blood glucose level before after the administration of STZ to check if type 2 diabetes is present.

2.6 Neurobehavioural Studies

2.6.1 Barnes maze

Barnes maze is a psychological laboratory experimental too used for measuring spatial learning and memory [30]. The function of a Barnes maze is to measure the ability of a rat to learn and remember the location of distal visual cues located around the testing is [29].

2.6.2 The plan for the Barnes’ maze was as follows

- **Day 1:** this day was used to make the Wistar rat to become familiar with the maze and to make it have an idea of what it entails.
- **Day 2:** training day 1, on this day a pelleted feed was kept in one of the holes and the rats were trained on how to find the box with the feed in it.
- **Day 3:** training day 2, this day was used for training but the animals were given a chance to find the box with the feed on their own, each rat was allowed to try severely until it could find the box with the feed.
- **Day 4:** this day was used to rest from the activity
- **Day 5:** this day was used as the probe day, the rats weren’t fed throughout and then they were placed on the Barnes maze and was watched closely to see if they will be able to locate the box with feed in it.

During this examination, the amount of seconds or minutes it took for the rats to locate the box with the feed, number of errors poked, primary and total latency were measured.

2.7 Animal Sacrifice

At the end of the administration, animals were sacrificed by cervical dislocation,
the head of the animal was cut open and brain removed. Prefrontal cortex was carefully dissected and fixed in 10% formal saline for histological analysis using:

1. Haematoxylin and Eosin stain for general histoarchitecture of the animal prefrontal cortex.
2. Cresyl fast Violet stains for Nissl substance.

3. RESULTS

3.1 Results on Weights Measured

During this research experiment, the weights of the animals were weighed weekly. The Fig. 1 shows the average weight of the animals per group during the experimental period.

3.2 Results from the Barnes Maze

Multiple comparisons were done between the primary latency travelled in barnes maze $P = 0.05$ the primary latency of Virgin coconut oil VCO (3.60±0.40) showed no significant difference when compared to control group (3.80 ± 0.37), there was a significant difference when diabetes Mellitus group DM (33.60±39.4) was compared to control group (3.80±0.37) there was no significant difference when treatment group (DM + VCO) (8.40±1.91) was compared with the control group (3.80±0.37), there was a significant difference when treatment group (DM + VCO) (8.40±1.91) was compared with diabetes Mellitus group DM(33.60±39.4).

![Fig. 1. Showing weight of the animals in different groups](image1)

**Fig. 1.** Chart showing the primary latency period of experimental animals in the Barnes maze

*Group A (control), Group B (VCO), Group C (dm), Group D (dm+vco)*
3.3 Histology Results

Histological analysis was carried out on each group with the application of histological stains at various magnifications, results were thus shown below:

Plate 1: Photomicrographs showing panoramic views of prefrontal cortex general histomorphology
Plate 1.1: Photomicrographs showing panoramic views of prefrontal cortex general histomorphology
Plate 2: Photomicrographs showing Nissl profiles of prefrontal cortex general histomorphology

4. DISCUSSION

In this research high-fat diet and streptozotocin was used to induce type 2 diabetes mellitus and virgin coconut oil was used as treatment. During this research weights of the animals were measured, Barnes maze was conducted as to test for cognitive function and histological analysis was also done.

Diabetes mellitus group lost a significant amount of weight, this result affirms that DM is associated with loss of weight, during diabetes there is a decrease in body weight as a result of the altered metabolic function as supported by Savitha et al. [30]. But in the Virgin Coconut oil group, the animals added a significant amount of weight which suggests that VCO helps improve appetite. The diabetes mellitus group had the highest latency in the barnes maze test, this suggests that they had a poor memory of the activity, while the Virgin Coconut Oil group had the lowest primary latency which affirms that virgin coconut oil enhances cognitive function. In the treatment group (dm +vco) the primary latency period was significantly reduced when compared to the dm group, this
Plate 1.1. Photomicrographs showing panoramic views of prefrontal cortex general histomorphological presentations in Wistar rats across the study groups. Hematoxylin and Eosin stain (X400). The Control and VCO groups (Group A & B) present normal neurons and glia cell without any mechanical assault (Black and Red arrows). Group C (STZ or DM) present neuronal degeneration or assault as represented by Pyknosis (Blue arrow) and Vacuolation (yellow arrow) Group D (STZ + VCO) present some normal neuron and glia cells (Black and green arrows) as well as abnormal or assaulted neurons and glia cells (Red and Blue arrows).

| Group A: control group |
| Group B: virgin coconut oil group (VCO) |
| Group C: Diabetes Mellitus group (DM) administered high-fat diet and streptozotocin |
| Group D: Diabetes Mellitus group (DM) administered high-fat diet and streptozotocin and virgin coconut oil group (VCO) |

Plate 1. Photomicrographs of Prefrontal cortex of Rat stain with H&E suggests that vco helps to improve the impaired memory and cognitive function in type 2 diabetes mellitus.

The histology of the prefrontal cortex of each group was examined using two stains: the H&E stain, and cresyl violet stain.

Normal histological features of the Control and VCO treated groups did not show any observable altered panoramic morphological presentation of the PFC layers (black arrows). From this study across the various exposures and magnification, the well-outlined array of cells within the PFC can be observed distinctly arranged from the Layer I-IV. In addition, cellular density within these groups appears normal across all the cortical layers with appreciable spines and neuronal projections (black arrows). STZ induced degenerative changes in the cortex and was characterized by fragmented pyramidal and granule cell layer with observable pyknotic cells (yellow arrows). Also, there appeared to be a comparatively increased cell density in the cortical layers of these treated groups. VCO treatments showed slight degenerative changes (yellow arrows) mildly similar to the morphologic appearance of STZ treatments; their cortical layers seem better structured and delineated with a distinct layering when compared with STZ groups.
Plate 2. Photomicrographs showing Nissl profiles of prefrontal cortex general histomorphological presentations in Wistar rats across the study groups. Cresyl fast violet stains (X100). The Control and VCO group (Group A&B) presented normal nissl profiles (Red arrows). Group C (group STZ or DM) showed serious loss of nissl substance (Yellow arrows) while Group D (STZ + VCO) showed moderate or fewer loss of nissl substance (Yellow arrow)

Group A: control group
Group B: virgin coconut oil group (VCO)
Group C: Diabetes Mellitus group (DM) administered high-fat diet and streptozotocin
Group D: Diabetes Mellitus group (DM) administered high-fat diet and streptozotocin and virgin coconut oil group (VCO)

Also, Nissl profile demonstration by CFV stain (X100) across PFC sections within the study groups shows poor staining outcome in the untreated STZ-induced diabetic rats compared with the control. The weak staining characteristic of the prefrontal pyramidal cells in these diabetic rats suggests loss or reduction of Nissl bodies of these cells; normal morphological presentations in CONTROL and VCO treatments that are characterized with normal and densely populated Nissl proteins, well stained and outlined neurons (Red arrows). STZ caused severe chromatolytic changes as well as some pyknotic changes in both the pyramidal and granule cell layers with a gross reduction in the cytoplasmic Nissl proteins (yellow arrows). And this implies decreased the synthetic activity of the pyramidal neurons, with possible impairment of cognitive function in these animals.

5. CONCLUSION

Diabetes affected the prefrontal cortex negatively while virgin coconut oil seems to neutralize the adverse effect and tried to restore the integrity of the prefrontal cortex. VCO has the potential to be used as a memory enhancer, the effect of which was mediated, at least in part, through enhanced cholinergic activity, increased antioxidants level and reduced oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was done in Babcock University and approved by the Babcock University Ethics
commission (BUHREC). The experiment took the total of 45 days.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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