Chinese Herbal Medicines 12 (2020) 414–420

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Long-term consumption of virgin coconut (*Cocos nucifera*) oil diet impairs learning and memory in CD1 mice

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ARTICLE INFO

Article history:
Received 22 May 2019
Revised 30 September 2019
Accepted 25 March 2020
Available online 15 August 2020

Keywords:
Cocos nucifera Linn
learning and memory
mice
virgin coconut oil

ABSTRACT

Objective: Virgin coconut oil (VCO) has been used in the management of dementia in Alzheimer's disease (AD). Therefore, this research investigated the effect of long-term consumption of VCO diet on learning and memory in CD1 mice.

Methods: Thirty male CD1 mice (divided into three groups, $n = 10$) were fed with standard rodent chow (control), 5% and 20% VCO diets (respectively) for 28 d. The Morris Water Maze (MWM) test was used to test the effect of VCO on visuo-spatial learning and memory, while the Novel Object Recognition Test (NORT) was used to measure short- and long-term recognition memory.

Results: Learning performance of mice did not differ in the MWM. During the probe trial, duration in the retention quadrant and annulus crossings were lower ($P < 0.05$) in the 5% and 20% VCO diet groups compared to the control diet group, showing that VCO impaired visuo-spatial memory. During the NORT, mice showed more total approaches in the 20% VCO diet group ($P < 0.05$) compared to control and the 5% VCO diet groups during the short-term memory test. During the long-term memory retention test, the total approaches were also higher in the 20% VCO group compared to control and 5% VCO group ($P > 0.05$). The discrimination index was also lower in the 20% VCO group compared to control and 5% VCO diet groups indicating impaired long-term cognitive memory in mice given 20% VCO diet. Histological examination of brains showed damage within the CA1 pyramidal cell layer of the hippocampus in the 20% VCO diet group, in line with the behavioural observations.

Conclusion: Long-term consumption of virgin coconut oil diet impairs memory in mice.

1. Introduction

Dementia is characterized by a gradual decline in cognitive functions, including memory, language, problem-solving and other skills that affect a person's ability to perform everyday activities. This decline occurs due to neuronal damage in brain areas responsible for cognitive function. One of the major neurodegenerative disorders that manifests as dementia is Alzheimer's disease (Barker et al., 2002; Wilson et al., 2012).

The burden of AD is quite high. In America alone, the Alzheimer's Association estimated that as of 2016, 5.4 million persons have Alzheimer's disease, 5.2 million of whom are age 65 and older. This translates to 1 in every nine people age 65 and older or 11 percent of this population having Alzheimer's disease (Alzheimer's Association, 2016; Hebert et al., 2013). The remaining number, approximately 200,000 individuals, are under age 65 and have early-onset Alzheimer's (Alzheimer's Association, 2016). In spite of these alarming statistics, it is still believed that a large portion of Americans may not know they have Alzheimer's disease because Alzheimer's disease is underdiagnosed and underreported. Subjective cognitive decline (SCD), a condition of worsening or more frequent confusion or memory loss, is often referred to as one of the earliest warning signs of AD. Even though not all cases of SCD will eventually develop to AD or other dementias, it still remains a high risk for AD and other dementias (Jessen et al., 2010; Kaup et al., 2015; Reisberg & Gauthier, 2008; Reisberg et al., 2010).

Although statistics on dementias in Nigeria and other sub-Saharan African (SSA) countries are not very accurate, the preva-

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https://doi.org/10.1016/j.chmed.2020.03.008
1674-6384/

lence of dementia seems lower when compared to developed countries such as United States of America. Bearing in mind age as the strongest risk factor for dementia, the low rates of dementia, are mostly attributed to the low life expectancy within these countries (Launer et al., 1999; Stephan & Brayne, 2008). For example, in some SSA population-based studies, the age-standardized annual incidence rate of dementia reported among the Yorubas in Nigeria was 1.35% (AD = 1.15%) (Winkler et al., 2011). In another study, however, the prevalence was higher, with 21.60% in the rural Hai district of Tanzania (Paddick et al., 2013). Although AD was the most reported dementia subtype in the Hai district (Ogunniyi et al., 1997; Paraïso et al., 2011; Yusuf et al., 2011). Other major risk factors for dementia were identified, including increasing age, female gender, cardiovascular disease, and low-literacy level (Gureje et al., 2011; Ogunniyi et al., 1997; Paraïso et al., 2011).

The above statistics have driven the search for a means to either delay the onset or prevent dementias. One such way that has been proposed is the use of coconut oil, acclaimed to have benefits for improving cognitive function in people with Alzheimer's disease (Jauregui, 2012; Johnson, 2012; Moore, 2008; Newport, 2008; Pope, 2011; Villariba, 2011).

Coconut oil is obtained from the mesocarp of fruits of the coconut palm (*Cocos nucifera* Linn). *C. nucifera* belongs to the family Arecaceae (Palmae), a large family of about 217 genera and about 2500 species. The palm of concern, *C. nucifera,* belongs to the order Arecales; And the genus *Cocos* belonging to the subfamily Cocoideae, comprising 27 genera, and 600 species (Evans, 2002). The basic crude constituents of the coconut oil include triacylglycerols (95% of its crude content), fatty acids (lauric acid being most predominant; 47.5%), phospholipids, tocopherols, steroids, trace metals, mono and diacylglycerols (Rossel et al., 1985). Coconut oil is commonly used particularly in South-South Nigeria for preparing a favorite delicacy, coconut rice.

Marked improvements have been reported anecdotally in Alzheimer's disease patients (Steve Newport), after consuming extra-virgin coconut oil with high levels of MCTs, vitamins, and related supplements for 37 d (Moore, 2008; Newport, 2008). Others have also reported that the intake of coconut oil led to improvement in cognition (Freeman et al., 2011; Granholm et al., 2008; Nevin & Rajamohan, 2004). Other research studies have suggested that coconut oil diet can provide symptomatic relief to a broad range of neurodegenerative disorders such as Parkinson's, Huntington's disease, traumatic brain injury, and stroke (Gasior et al., 2006) and can even improve memory in neurodegenerative diseases Fernando et al., 2015). In cortical neurons treated with amyloid-β (Aβ) peptide *in vitro*, co-treatment with coconut oil reportedly increased neuronal survival compared in cultures exposed to Aβ alone (Nafar & Mearow, 2014). Other medicinal properties of the oil are its analgesic, antipyretic, anti-inflammatory, antimicrobial and anti-toxic effects (Intahphuak et al., 2010).

Despite the benefits ascribed to coconut oil, there are still some controversies concerning the positive effects of the oil. For instance, high calorie daily doses of MCT oil and non-hydrogenated extra-virgin coconut oil (a high ketone diet) for more than two or three years may increase the risks for negative effects such as acidosis, hypocalcemia, hyperlipidemia or carcinogenesis (Bergqvist, 2011; Maalouf et al., 2009; Shilhavy, 2012). Some researchers have also linked coconut oil to higher levels of LDL, high content of saturated fatty acids, and a higher risk for cardiovascular disease (Dietschy & Turley, 2001; Laureles et al., 2002). Therefore, the motivation for this study was to investigate the effect of long-term consumption of coconut oil diet on learning and memory in mice, as a way of examining the potential benefits and risks of coconut oil for treating cognitive impairment in dementia.

The aim of this study was to investigate the effect of long-term consumption of virgin coconut oil diet on learning and memory in mice. Therefore, if under normal conditions, the VCO could improve memory, it would possibly be applicable in a case of AD type dementia. This was accomplished by examining how long-term consumption of either 5% or 20% VCO diets affects visuo-spatial learning and memory in the Morris water maze test, and short- and long-term recognition memory in the Novel Object Recognition Test.

2. Materials and methods

2.1. Design

Thirty inbred male CD1 mice (body weight: 16.5–22.6 g), obtained from the animal house of the Department of Physiology, University of Calabar, were used for the experiment. Mice were housed five per cage of dimensions 45 cm × 30 cm × 25 cm and a 12/12 light/dark cycle. The mice were divided into three groups (n = 10). Control mice were fed with normal rodent chow (vital feed; growers mash) while the other mice received either 5% and 20% virgin coconut oil diets respectively for 28 d. All animals had clean drinking water *ad libitum* (Fragua et al., 2015; Jayachandran et al., 2015).

2.2. Preparation of virgin coconut oil

Coconut oil was prepared using the wet mill method of Hamid et al. (2011) as modified by Bisong et al. (2017). Coconut oil was extracted from the kernel (cotyledon) of mature freshly harvested fruits. Water (100 mL) at room temperature was added to 500 g of the grinded coconut kernel, and the coconut milk was extracted using a fine mesh. The extracted coconut milk was kept at room temperature of about 25 °C and allowed to ferment for 24 h and then frozen for 15–20 h. When allowed to defrost, the milk separated into three layers (chaff, coconut oil and water respectively). The virgin coconut oil, was used to constitute 5% coconut oil diet (i.e. 95 g growers mash and 5 g VCO) and 20% coconut oil diet (80 g standard growers mash mixed with 20 g VCO).

2.3. Behavioral assay

The Morris Water Maze (MWM) apparatus was used to assess visuo-spatial learning and memory (McDonald & White, 1994). The MWM consisted of a circular pool (110 cm diameter) filled with opaque water. The first 3 d consisted of acquisition training, during which the mice would swim to locate a hidden escape platform. Following acquisition training, mice then completed reversal training for 3 d, where the escape platform was moved to the opposite side of the maze. On day 7, mice completed a 60-sec probe trial to assess memory. During the probe trial, the escape platform was removed and mice were allowed to explore the maze. The duration in each quadrant and annulus crossings were recorded. Day 8 had four trials with a clearly marked visible platform (Bisong et al., 2016). The Novel Object Recognition Task (NORT) was completed in an open box (38 × 38 × 38 cm) with a central square of 15 × 15 cm. Objects were placed within the central square to assess recognition memory. The test procedure consisted of an initial 2 min habituation trial, followed by a 5 min acquisition trial (trial 1) and a 5 min retention trial (trial 2). These two trials were separated by a retention period (inter-trial interval): 15-minutes for a short-term memory test and 24-h for a long-term memory test. In trial 1, two similar objects were placed at the diagonal ends of the central square, and each mouse was allowed to explore the objects. During the retention trial, one familiar object was replaced

with an unfamiliar/novel object. Frequency and duration of approach to each object was scored. The index of discrimination (d) was calculated by the formula:

d = (time spent exploring novel object – time spent exploring familiar object)/ (time spent exploring novel object + time spent exploring familiar object) [43,44].

2.4. Histological examination of hippocampus

Standard Haematoxylin and eosin staining were employed to stain sections of the hippocampus for observation. Prepared sections were mounted, and viewed at × 400 magnification with a microscope.

2.5. Statistical analysis

The One-way analysis of variance (ANOVA) was used to analyse data and Post-hoc Newman Keuls test was used to analyse for statistical difference in pairs of groups. Results were presented as means ± standard errors of means (SEM) and the probability level $P < 0.05$ was accepted as significant. The statistical packages Microsoft Excel 2010 and SPSS 18.1 were used for data analysis.

Ethical approval

Ethical approval for this research was obtained from the Animal Ethics Committee of the Faculty of Basic Medical Sciences University of Calabar, Calabar Nigeria, with protocol number 015PY20216 issued in February 2016.

3. Results

3.1. Effect of VCO on visuo-spatial learning and memory in MWM

The learning curves for swim latencies to locate the escape platform for the control, 5% and 20% coconut oil diet during acquisition training (Fig. 1A), did not differ between the groups. Similarly, the learning curves during the reversal training (Fig. 1B) did not differ significantly between the 5% coconut oil diet and control. However, swim latencies for the 20% coconut oil diet were significantly longer than both control and the 5% diet group ($P < 0.05$). Thus, the 5% coconut oil diet-fed group of mice showed a normal learning curve whereas there was impaired reversal learning in the 20% coconut oil diet-fed group particularly on the first day of reversal training.

During the probe trial, duration in the retention quadrant for the control, 5% coconut oil diet and 20% coconut oil diet groups were (19.73 ± 1.20) s, (16.61 ± 1.35) s and (15.08 ± 1.47) s respectively (Fig. 2). The retention quadrant duration was significantly lower in the groups of mice fed on 5% and 20% coconut oil diet ($P < 0.05$ and $P < 0.01$ respectively) when compared to control. The frequency of annulus acquisition crossings did not differ between mice fed on 5% coconut oil diet and control. However, the frequency of annulus crossings at the acquisition escape location was significantly lower in the group of mice fed on 20% coconut oil compared to the control group and 5% coconut oil diet groups ($P < 0.05$). The frequency of crossings at the reversal escape location was also significantly lower in both test groups (5% and 20% coconut oil diets groups) when compared to control mice ($P < 0.05$) but did not differ between each other (Fig. 3).

Fig. 4 showed comparison of the swim latency between the test groups and control mice during the visible platform task. The swim latencies for the control, 5% coconut oil diet and 20% coconut oil diet groups were (10.875 ± 1.55) s, (13.258 ± 1.97) s and (13.257 ± 1.80) s respectively, and did not differ significantly between groups.

3.2. Effect of VCO on recognition memory in NORT

During the short-term memory test, the total number of approaches to objects did not differ between mice fed with 5% coconut oil diets (Fig. 5A) and control mice, but it was significantly higher ($P < 0.05$) in the group of mice fed with 20% coconut oil diet compared to control mice and mice fed with 5% coconut oil diet ($P < 0.05$). The index of discrimination for short term-memory did not differ significantly between the groups (Fig. 5B). The total number of approaches to objects and the index of discrimination in the long-term memory test of the novel object recognition task (Fig. 6) also did not differ between 5%, 20% coconut oil diet and control.

3.3. Effect of VCO diets on histology of hippocampus CA1 cell layers using H&E staining

Photomicrographs of sections through the hippocampus of the three groups of animals are presented in Fig. 7. At magnification × 400, the photomicrographs clearly showed a distortion in the pyramidal cell layer of the mice treated with 20% virgin coconut oil diet (Fig. 7C) when compared to the normal appearance of mice receiving control (Fig. 7A) or 5% VCO diets (Fig. 7B).

Fig. 7A is a section of the hippocampus from mice receiving control diet, showing the molecular layer, the pyramidal cell (PYC) layer and the stratum oriens (polymorphic layer). The molecular cell layer shows sparsely populated neuronal cell bodies (NCB) and neuronal processes while the pyramidal cell layer show

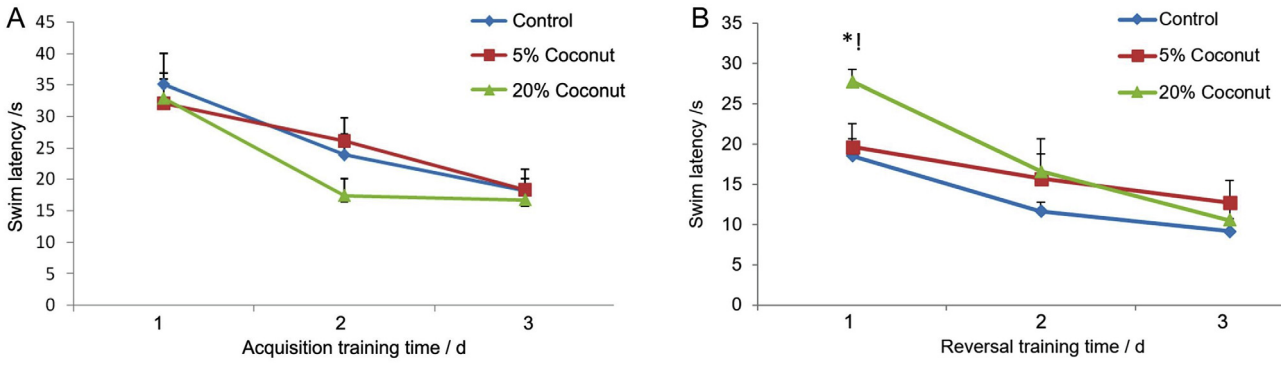

Fig. 1. Comparison of learning curves (showing swim latencies in seconds) during acquisition training (A) and reversal training (B) of Morris water maze test for mice fed on 5% and 20% coconut oil diet. * – Significant at $P < 0.05$ *vs* control; ! – Significant at $P < 0.05$ *vs* 5% coconut oil diet group.

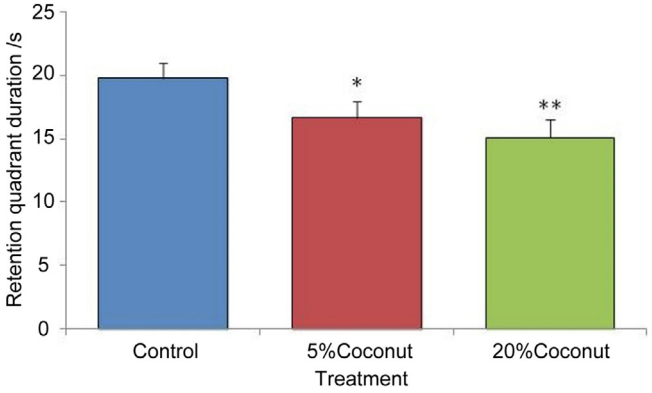

Fig. 2. Comparison of retention quadrant duration in probe trial of Morris water maze test for mice fed on 5% and 20% coconut oil diet. * – Significant at $P < 0.05$; ** – Significant at $P < 0.01$.

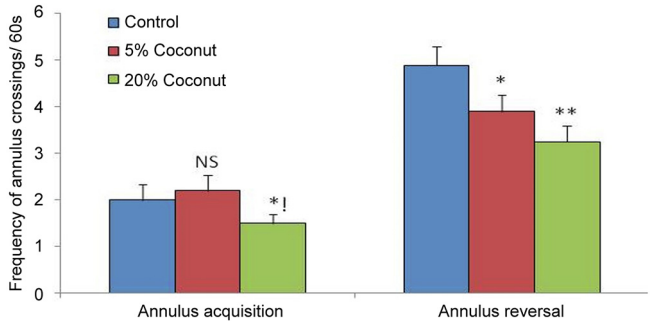

Fig. 3. Comparison of frequencies of annulus acquisition and annulus reversal crossing in probe trial of Morris water maze test for mice fed on 5% and 20% coconut oil diet. NS – Not significant compared to control; * – Significant at $P < 0.05$; ** – Significant at $P < 0.01$; ! – Significant at $P < 0.05$ compared to 5% coconut diet fed on mice.

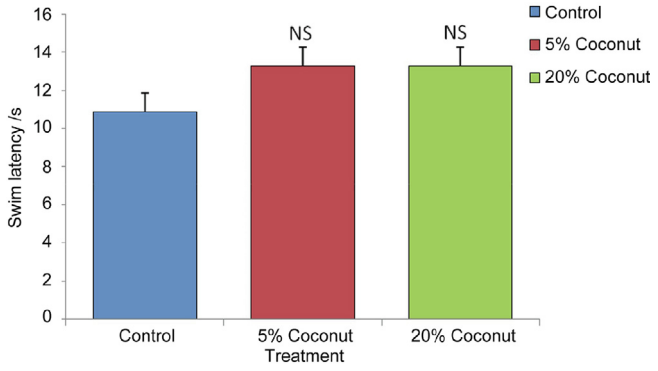

Fig. 4. Comparison of swim latency during visible platform task of Morris water maze test for mice fed on 5% and 20% coconut oil diet. NS – Not significant compared to control.

densely populated pyramidal cells with triangular shape bodies, prominent nuclei. The polymorphic layer consists of abundant neuropil comprised of neuronal cell processes and neuroglia. These layers appeared normal without any distortions.

Fig. 7B is a section of the hippocampus from mice receiving 5% VCO diet showing the outer molecular cell layer, intermediate pyramidal cell layer and inner polymorphic cell layer. Similarly, the pyramidal cell layer consists of prominent pyramidal cells of various sizes with triangular shaped bodies having prominent nuclear and nucleoli; And the molecular and polymorphic cells all showing no visible damage.

Fig. 7C is a section of the CA1 area of the hippocampus from mice receiving 20% VCO diet showing prominent cell layers, molecular layer, intermediate pyramidal and the inner polymorphic cell layers. However, the pyramidal cells layer showed sparsely populated cells with poorly stained nuclei, indicating cellular disintegration. The cells in this layer were less densely populated when compared to their other counterparts, showing a clear damage to the cells.

4. Discussion

The effect of long-term consumption of 5% and 20% virgin coconut oil diets on learning and memory was examined using the Morris water maze (MWM) and the novel object recognition task. The hidden-platform version of the Morris water maze, which is hippocampus-dependent, was used to test visuo-spatial learning and memory (McDonald & White, 1994). The swim latencies during acquisition and reversal training were recorded to examine acquisition and reversal learning, respectively. As the trials were repeated over 3 d, swim latencies got shorter, indicating that the mice learned to use extra-maze cues to locate the escape platform, and indicated that learning occurred in all the groups equally well. This trend was also observed for the reversal training except for the reversal day 1, where the 20% diet group had longer swim latencies showing an initial delay in relearning of the hidden platform position.

The probe trial conducted on day 7 of the MWM test showed reduced duration in the retention quadrant in both the 5% and 20% virgin coconut oil diet groups of mice. Similarly, the annulus reversal crossings were lower in both coconut diet groups and annulus acquisition crossing for the 20% diet group. Mice with good memory of the position of the hidden platform during the reversal training should spend more time searching in the quadrant which had the platform during reversal training (retention quadrant) before searching elsewhere (mostly in the quadrant with the platform during acquisition). These mice would also do more annulus crossing there. Conversely, mice with impaired memory would spend less time in the retention quadrant and do less annulus crossings. Thus, the mice fed with coconut oil diets (especially those fed 20% diet) showed impaired visuo-spatial memory.

The novel object recognition task (NORT) is based on the principle that rodents will spend more time investigating a new (novel) object than they would a familiar one (Brown et al., 1999; Podhorna & Brown, 2001; Sik et al., 2003). Therefore, if a mouse recognises a familiar object, it would spend less time investigating it. This test has an advantage over the MWM because it creates less stress and thus stress is less likely to interfere with the learning and memory performance on the NORT (Sik et al., 2003). The results during the retention trial of the NORT showed increased total number of approaches to objects in the 20% diet group compared to control mice (even though the 5% diet group did not differ) both in the short-term and long-term memory tests. Based on the principle of the test, it was expected that the animals are already familiar with one of the objects; Therefore, the total approaches were not expected to be high for mice that remember the familiar object. The index of discrimination is a function of the preference the animal has for the novel object. Mice that remember the familiar object will have a higher index of discrimination hence improved memory, and *vice versa*. Results here showed that the index of discrimination was lower for the mice fed 20% coconut oil diet, for both short-term and long-term memory tests, indicating impairment in both short-term and long-term recognition memory. Therefore, long-term consumption of 20% virgin coconut oil diet also impaired recognition memory in the mice. Although

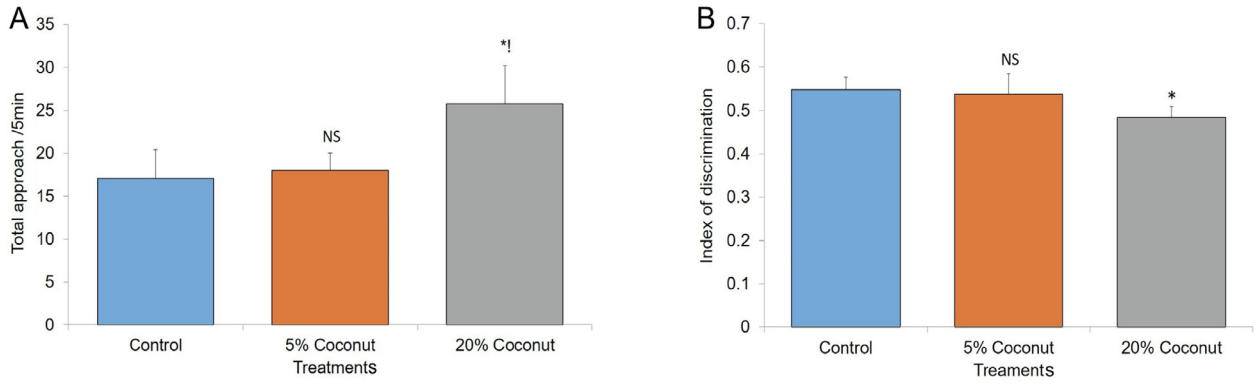

Fig. 5. Comparison of total number of approaches to objects (A) and index of discrimination (B) during retention trial of novel object recognition task for short term memory assessment in mice fed on 5% and 20% coconut oil diets. NS – Not significant compared to control; * – Significant at $P < 0.05$ *vs* control; ! – significant at $P < 0.05$ *vs* 5% coconut diet.

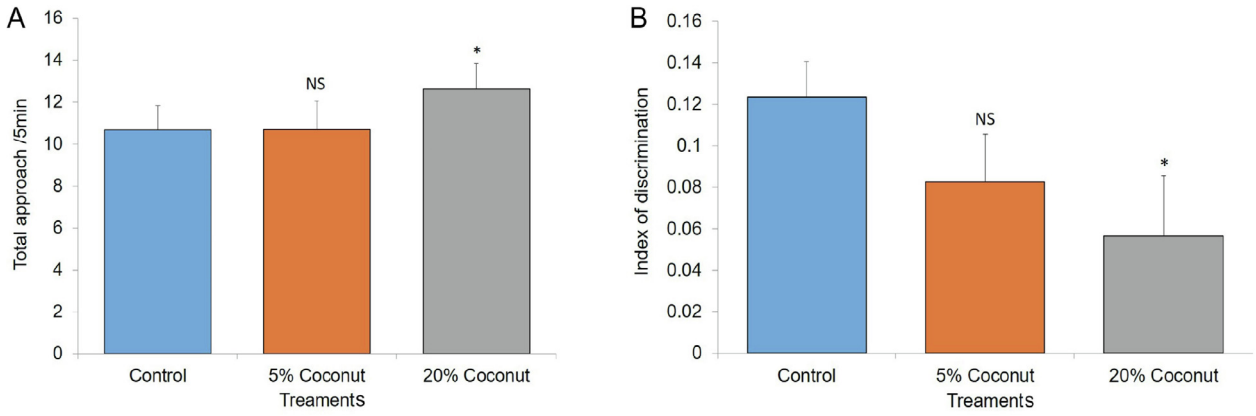

Fig. 6. Comparison of total number of approaches to objects (A) and index of discrimination (B) during retention trial of novel object recognition task for long-term memory assessment in mice fed on 5% and 20% coconut oil diets. NS – Not significant compared to control; * – significant at $P < 0.05$ *vs* control.

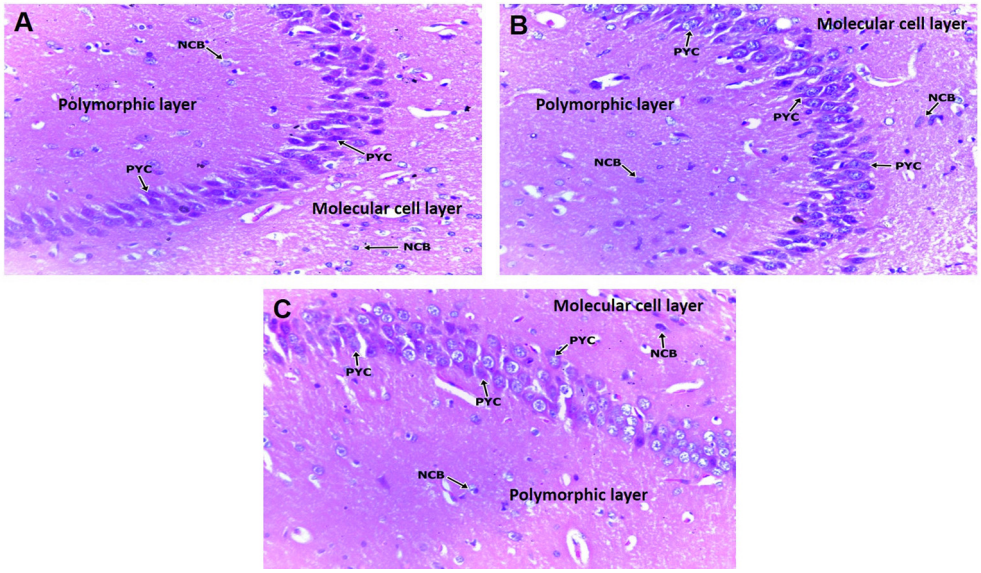

Fig. 7. Photomicrographs of hippocampus of control mice (A), mice fed on 5% virgin coconut oil diet (B) and 20% virgin coconut oil diet (C) (magnification: ×400).

this was not significantly observed for the 5% VCO diet group of mice, it showed a trend towards impairment of memory.

The results for the histology of the hippocampus similarly showed a clear degeneration of the pyramidal layer in CA1 region of the hippocampus in the group of mice fed with 20% virgin coconut oil diet. This corroborates the results in the MWM and NORT which showed impaired visuo-spatial memory and cognition memory in the 20% virgin coconut oil diet group of mice.

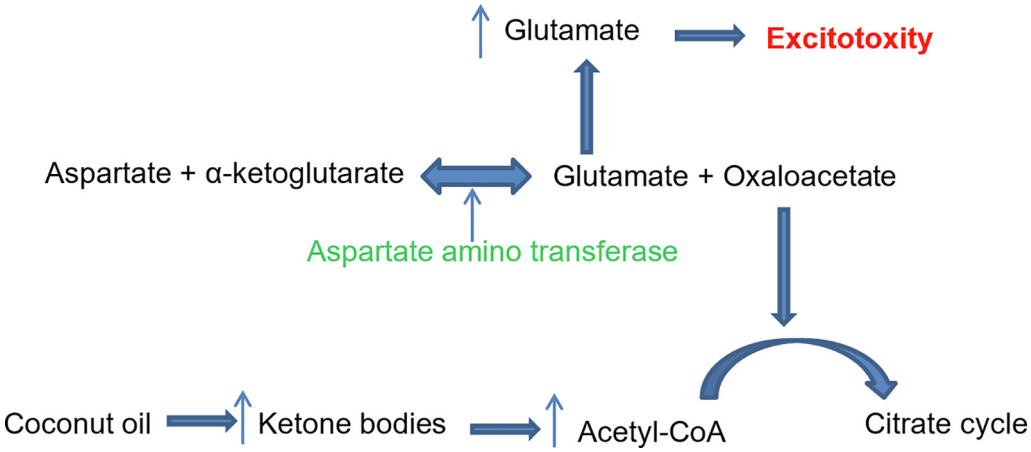

Fig. 8. Possible mechanism by which long-term consumption of coconut oil diet causes memory impairment.

The hippocampus is critical for the formation of memory. One of the groups of cells that form the major excitatory output of the hippocampus is the pyramidal cells. These pyramidal cells – particularly the pyramidal cells of the CA1 area of the hippocampus – play a very important role processing of both spatial and non-spatial information that is vital for memory formation (Graves et al., 2012). These pyramidal cells of the CA1 area of the hippocampus form the major point of origin for long-term potentiation, which is the basis for long-term of memory formation Wozny et al., 2008). Therefore, it is clear that the observed degeneration in these cells is responsible for the impaired memory observed in the behavioural tests used in the present study.

The results of this study contradict the research of Reger et al, (2004) whose research reported that those who drank a beverage with the medium chain triglycerides (MCTs) from coconut oil scored significantly better on cognitive tests and therefore had improved memory. It also contradicts the research of Van der Auwera et al. (2005) which reported that ketogenic diet such as that from coconut oil reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease.

Indeed, it is possible that virgin coconut oil could improve memory in cases of Alzheimer's disease. Under normal circumstances, the brain uses glucose as the major energy substrate. However, in neurodegenerative disorders such as Alzheimer's disease, glucose metabolism to produce energy is diminished in the brain resulting in the phenotypes among which are cognitive decline (Fife, 2016); Hence the need for an alternative source of energy for the brain. An alternative source of energy for the brain is the ketones or ketone bodies. Virgin coconut oil has a high content of medium chain triglycerides (MCTs), which can be easily converted by the liver to ketones (ketogenic diet). Since VCO is a ketogenic diet, its consumption would result in increased availability of ketone bodies for brain metabolism, thereby tentatively restoring the brain function and improving memory.

Based on this, ketogenic diets have been reported in some initial studies to have provided symptomatic relief to a broad range of neurodegenerative disorders such as Parkinson's disease, ALS, Huntington's disease, traumatic brain injury and stroke (Duan et al., 2003; Gasior et al., 2006; Tieu et al., 2003; Vanitallie et al., 2005; Zhao et al., 2006). Similarly, in animal models of Alzheimer's disease, ketogenic diets have been reported to reduce the amount of Alzheimer's-like plaques that form in the brain and improved performance on visual-spatial memory tasks, increase the ability of learning tasks, and improve performance in short-term memory tests (Costantini et al., 2008; Reger et al., 2004; Van der Auwera et al., 2005).

It is not clear why the results of the current study negate earlier results on coconut oil and memory. We agree that while ketone bodies from virgin coconut oil may be beneficial in small quantities administered over short periods, they may have negative effects if used in large quantities over prolonged periods. This data is consistent with the hypothesis that the metabolism of ketone bodies to acetyl-CoA results in a diminution of the pool of brain oxaloacetate (Daikhin & Yudkoff, 1998). We therefore hypothesize a possible mechanism by which long-term consumption of coconut oil diets would cause memory impairment (Fig. 8). Coconut oil is ketogenic and so long-term consumption of this oil would result in large amounts of ketones generated in the system. The metabolism of ketone bodies results in an increase in the generation of acetyl-CoA. Naturally, aspartate combines with alpha ketoglutarate to form glutamate and oxaloacetate in a reversible reaction catalysed by the enzyme aspartate aminotransferase. The high levels of acetyl-coA generated from the ketones increases its combining with Oxaloacetate in the citrate synthetase reaction of the citrate cycle (oxaloacetate + acetyl-CoA > citrate), resulting in decreased brain oxaloacetate. As less and less oxaloacetate becomes available to the aspartate aminotransferase reaction, there will be lowering of the rate of glutamate transamination, leading to increased glutamate levels in the brain. Increased brain glutamate levels therefore lead to excitotoxicity hence memory impairment. This mechanism however needs to be confirmed in additional research.

5. Conclusion

In conclusion, long-term consumption of coconut oil diet (particularly 20% coconut oil diet) impaired visuo-spatial and recognition memory in CD1 mice. If these results extend to humans, then prolonged consumption of this oil may worsen symptoms in neurodegenerative disorders like Alzheimer's disease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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