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Omega 3 Fatty Acid and Cannabidiol Prolong Lifespan and Ameliorates Brain Ischaemia in Mice Fed Chronic High Fat Diet

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Abstract

Diets these days contain more fats. High fat diet (HFD) is a model of unhealthy eating in experimental animals. It is known to induce inflammatory responses and oxidative stress. Cannabidiol (CBD), the non-psychoactive component of *Cannbis sativa* has been legalized for medicinal use in many countries of the world. Omega 3 fatty acid, commonly found in fish oil is medicinal and necessary for brain development. The antioxidant and neuroprotective functions of CBD and omega 3 have made them relevant in many researches. In this study, 45 mice were used and divided into three groups of 15 animals each: Group 1 (normal feed and water *ad libidum*); Group 2 (HFD *ad libidum*); Group 3 (HFD + CBD + Omega 3) for 16 weeks. Thereafter, five animals from each group were selected and their frontal lobes were harvested for histological analyses using H and E staining. The remaining mice were allowed to feed on normal diet and observed till death. At the end, HFD significantly reduced life span (57.4 ± 0.3) when compared to control (78.9 ± 1.6) and HFD + CBD + omega 3 group (74.5 ± 0.8) at $p < 0.05$. HFD also caused significant brain ischemic damage, neuronophagia and significant perivascular oedema. CBD + Omega 3 induced significant astrocytosis compared to control and HFD group. The immune stimulation by the CBD + Omega 3 could be responsible for tissue survival and longevity by protection from inflammatory and oxidative injuries. Ischaemic tissue death could have been prevented by amelioration of artheroma formation due to HFD. Further studies will be required to ascertain other possible mechanisms behind these findings.

Keywords

Omega 3, CBD, HFD, Lifespan, Astrocytosis

1. Introduction

Our world is undergoing a dietary transition as foods are becoming richer in fatty and caloric contents. This drastic western diet globalization is eroding healthy traditional feeding cultures around the world [1]. This has led to a significant rise in obesity and all its attendant health risks and complications [2]. High fat diet has been found to cause obesity in rodents [3] [4] and reduction in life span as well [5]. It has also been observed to reduce life expectancy in drosophila melanogaster [2].

Research has shown that consumption of foods containing bioactive dietary compounds promotes longevity and health span via certain epigenetic modifications like DNA methylation, histone modification and non coding RNA [6]. CBD is the non-psychotropic component of cannabis sativa, a plant used for various medicinal purposes from ancient times [7]. Its rich bioactive composition makes it a potential agent in immune modulation, anti-inflammation, anti-neoplastic and anti-aging processes as it influences the endocannabinoid system via the cannabinoid receptor [8]. Omega 3 is a bioactive lipid commonly found in fish oil and known to possess anti-inflammatory, cardio-protective and immune modulatory functions [9] [10].

The literatures on the effect of HFD on longevity are controversial. Some research findings show that it promotes longevity while others prove the reverse [11]. The use of CBD and omega 3 as a resource for boosting longevity in experimental HFD-fed mice is nonexistent in literatures. This research seeks to find that out.

2. Materials and Methods

2.1. Animals and Management

45 Swiss mice where purchased from the animal house of the College of Health Sciences, Benue State University (BSU), Makurdi. They were housed in plastic cages and allowed a 2 week acclimatization period under a 12 hour day/light cycle. They were divided in 3 groups of 15 animals each as follows:

Group 1: control group (feed and water *ad libidum*)

Group 2: HFD only *ad libidum* for 16 weeks)

Group 3: HFD + CBD (10 mg/kg/day) + Omega 3 (200 mg·kg/day) for 16 weeks

Thirty (10 from each group) of the mice were allowed to live on normal diet and water *ad libidum* till natural death. Five from each of the groups were anaesthetized with chloroform in a closed chamber and then sacrificed. Craniotomy was carried out and their brains (frontal lobes) were preserved in 10% formaldehyde. H and E staining was done on the brain specimen according to the protocol described by Geoffrey and Cindy (2020) [12]. This involves: dewaxing, dehydration, haematoxylin stain, bluing, eosin stain, dehydration, clearing and cover-slipping.

2.2. Composition of HFD

HFD was composed in the Physiology lab of the BSU, according to Julia *et al.*

(2018) [13] with some modifications. Total caloric content from fat (saturated and unsaturated) was 68.5% and lard was replaced with tallow.

3. Results

3.1. Effect of HFD on Life Span and Brain Histology

High fat diet significantly reduced lifespan of mice (Figure 1) compared to the control and the group supplemented with CBD and omega 3. Median life expectancy for HFD group (57.4 ± 0.3) was found to be significantly lower compared to control (78.9 ± 1.6) and HFD + CBD + omega 3 group (74.5 ± 0.8) at $p < 0.05$. HFD also caused significant brain ischemic damage, neuronophagia and significant perivascular oedema.

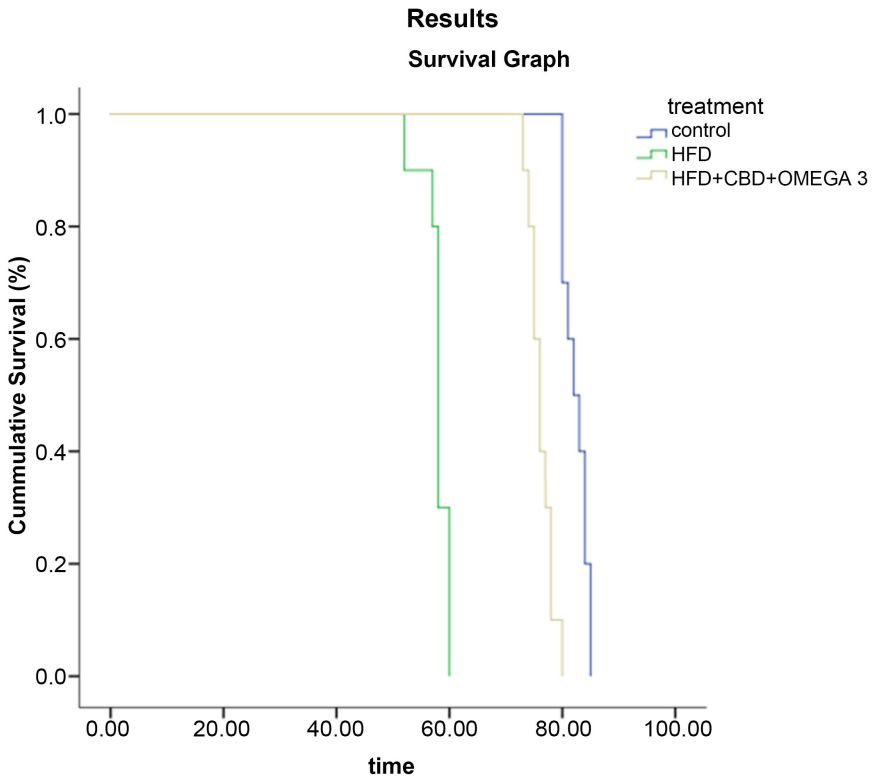

Figure 1. Kaplan-Meier survival curve showing the survival time of all groups of animals. The median survival time for the control, HFD and HFD + CBD + Omega 3 groups was 78.9 weeks, 57.4 weeks and 74.5 weeks respectively.

3.2. Effect of CBD + Omega 3 on Lifespan

Cannabidiol and omega 3 significantly prolonged lifespan of the mice (Figure 1) when given alongside the HFD. Cannabidiol and omega 3 also induced significant astrocytosis when compared to control and HFD group and prevented all the evidences of ischaemic damage.

3.3. Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. The differences

between the groups were analyzed by the Kaplan-Meier survival curve using the log rank, breslow and tarone-ware statistical test tools in SPSS version 20. Values of $p < 0.05$ were considered significant.

4. Discussion

CBD and Omega 3 increased life span of HFD fed mice by 17.1 weeks. According to Sulagna and Pullav (2016) [14] one human year is equivalent to nine mice days. This implies that long term consumption of unhealthy HFD can result in reduction of life expectancy by 13.3 years in humans. The shortening of lifespan by HFD has been reported in some literature, though the mechanism by which it shortens lifespan is still unclear. For instance, a meta-analysis study by Sara *et al.* (2018) [15] showed that mortality was heightened when animal based fat was substituted for carbohydrate or plant based proteins in a human population wide study. Most deaths were associated with cardio-metabolic complications. It is hypothesized that HFD stimulates inflammatory pathways, biological aging and oxidative stress. We found that CBD and Omega 3 ameliorated the effects of the HFD (**Figure 2**) by inducing astrocytosis, preserving brain morphology and prolonging lifespan. To support our findings a recent study has shown that down regulation of genes related to neural excitation and up-regulation of genes involved in immune function are strongly associated with promotion of longevity. Similarly, genes that protect against toxic stressors like reactive oxygen species and amyloid beta proteins are able to prolong lifespan [16]. Astrocytes are necessary immune cells in the brain that protects it from harmful agents. They are relevant homeostatic tissues that possess angiogenic, immunomodulatory, neurogenic and antioxidant properties [17]. It implies that the induction of astrocytosis by CBD and Omega 3 is necessary for brain and tissue survival. Their ability to dampen inflammatory and oxidative damage in other body parts like in the brain, may be responsible for the longevity seen in our experiment.

HFD on the other hand induced significant neuronal ischemic necrosis, perivascular oedema and neuronophagia (**Figure 3**) when compared to control (**Figure 4**). The theory of ischemia is basically related to severe stenosis or occlusion

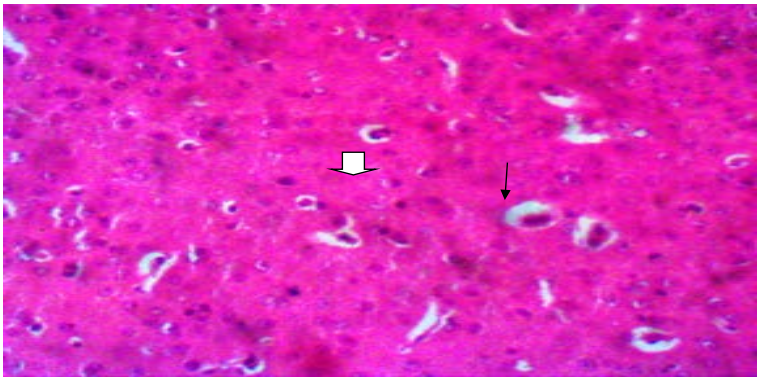

Figure 2. H and E staining of brain in the HFD group shows evidence of mild perivascular oedema (black arrows) and significant astrocytosis (white arrow). ×400 magnification.

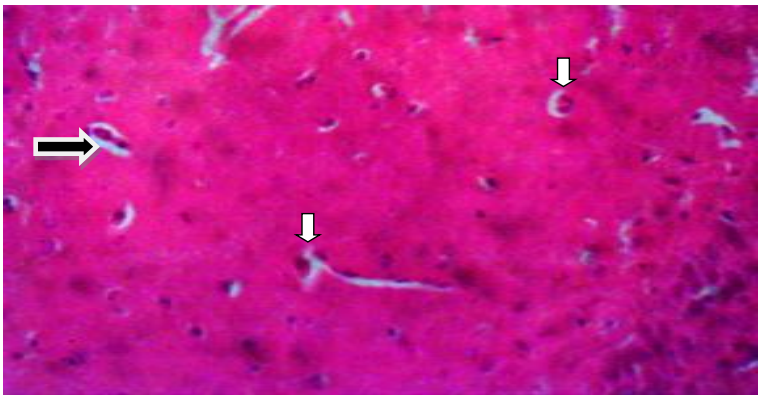

Figure 3. H and E staining of brain in the HFD group shows evidence of severe ischaemic neuronal necrosis (black arrows), neuronophagia and perivascular oedema (white arrow). ×400 magnification.

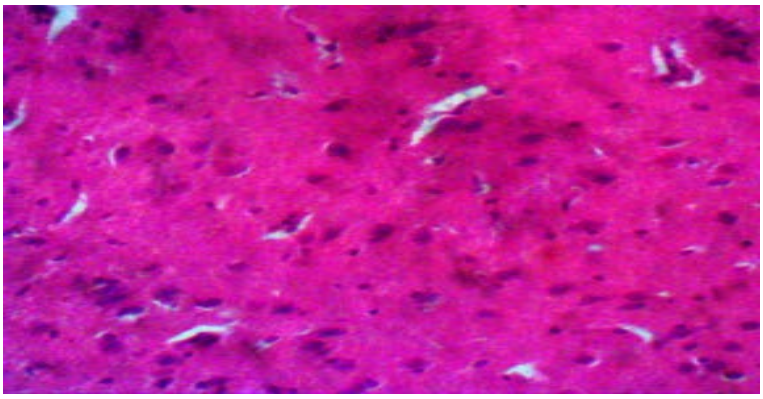

Figure 4. H and E staining of brain in the control group shows no evidence of lesion normal architecture. ×400 magnification.

of a cerebral artery, thus depriving the brain of glucose, oxygen, lipids and other vital nutrients leaving it necrotic. Neuroinflammation also occurs in the necrotic brain tissues due to a breach in the blood brain barrier and infiltration by peripheral inflammatory mediator cells [18]. HFD increases risk of artherosclerosis and induces inflammatory response causing widespread tissue and brain damage that result in shortening of lifespan. It also induces widespread production of reactive oxygen species [19] [20]. The CBD and Omega 3 significantly ameliorated these adverse outcomes in this study causing prolongation of lifespan.

5. Summary and Conclusion

HFD is detrimental to the survival of both brain and body tissues. It resulted in significant reduction in life span, induction of oxidative damage and reactive neuroinflammation. CBD and Omega 3 caused a prolongation of the lifespan in mice by induction of a potent immune response-astrocytosis and attenuation of neuroinflammation and other resultant tissue damage. It is quite necessary that chronic consumption of western diet, rich in fat and calories be discouraged in other to boost life expectancy and aid survival.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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