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High Fat Diet Alteration of Gut Microbiota Impacts Learning, Memory and Anxiety Response in Mice: Cannabidiol and Omega 3 Possible Remedies

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Abstract

Chronic intake of High Fat Diet (HFD) has the potential of causing a number of metabolic disorders. It is also theorized to be involved in perturbation of gut microbiota. Cannabididol (CBD) and omega 3 are known to possess a number of medicinal usefulness. Their combined use in experimental interventions is quite limited. A total of 15 mice were used for this research divided into three groups of five animals each. Group 1 was administered water and normal chow *ad libidum.* Group 2 had HFD and water *ad libidum.* Group 3 had HFD plus CBD (10 mg/kg) and omega 3 (200 mg/kg) all for a total of 12 weeks. They were tested on the elevated plus maze (EPM) and average entry time into the closed arm was recorded. They were also tested on the Y-maze and spontaneous alternations were measured. Thereafter animals were sacrificed and faecal content in the caecum was collected in sterile bottles and cultured for *E. coli* count. It was found that HFD group at p value < 0.05 had a significantly shorter closed arm entry time (22.5 ± 2.5) compared to control (80 ± 1.5) and CBD + omega 3 group (78 ± 2.0). Percentage alternations in group 3 were significantly higher (40 ± 3.3) compared to control (30 ± 2.8) and group 2 (28 ± 2.5). Group 2 had a significantly higher *E. coli* count ($2.4 \times 10^6 \pm 4.5$) compared to group 1 ($1.4 \times 10^6 \pm 5.6$) and group 3 ($1.42 \times 10^6 \pm 6.3$). The findings revealed that HFD enhanced gut *E. coli* overgrowth which was reduced by CBD and Omega 3. The memory impairment and anxiety induction by HFD was also significantly ameliorated by CBD and omega 3. *E. coli* known to be implicated in dementia induction was suppressed by the interventions. Possible mechanisms proposed are actions of CBD and omega 3

on CB1, TRVP and 5HT receptors in reducing anxiety and their antioxidant/anti-inflammatory actions in mitigating the neuro-inflammatory effect of HFD and immune hyperstimulation of *E. coli* via the gutbrain-axis.

Keywords

HFD, Learning, Memory, CBD, Omega 3, Anxiety, EPM

1. Introduction

The various systems of humans and mice are colonized by billions of microorganisms. Most of these microorganisms (bacteria, viruses and fungi) are found in gastrointestinal (GI) tracts and they are collectively called the gut microbiota [1] [2]. A strong relationship has been drawn between the nervous and the GI systems via the gut-brain axis [3]. The gut-brain axis (GBA) is a highly complex interactive network between the gut and the brain, composed of endocrinological, immunological and neural mediators [4]. Pertubations in the bidirectional gut-brain microbiota interactions have been implicated in the pathophysiology of certain neurobehavioural disorders like autism, depression, dementia, Parkinson's disease and schizophrenia [5] [6]. The gut microbiota is also able to influence vagal nerve activity, regulate stress response, amino acid metabolism and immune functions control. Dysbiosis of the gut microbiota has been found to induce atypical immune signaling; disruption in host homeostasis and various brain diseases [2] [7]. The early colonization of the gut by microbiomes is a necessity for the normal function of the brain such as the integrity of the blood brain barrier and synaptic plasticity [8].

Diets are very relevant in the balance of the gut microbiome. Chronic high fat diet in rodents has been shown to alter the genetic composition and the metabolic activity of the gut microbiota [9]. Recent researches have shown that high fat diet can cause intestinal dysbiosis, bowel inflammation, decreased expression of antimicrobial peptides, mucus layer depletion, barrier disruption and passage of bacterial particles into the blood stream with activation of secondary immune response, resulting in metabolic complications [10]. Also recently, it has been hypothesized that gut microbiomes can cause dementia illness such as Alzheimer's disease [11].

There are inconsistencies in the existing literatures regarding the effect of high fat diet on the gut microbiomes and this has been speculated to be probably due to the varying adaptation of the microbiomes [12]. We seek to add to the body of knowledge on how high fat diet affects gut microbes vis-à-vis the brain function.

2. Materials and Methods

2.1. Animals

A total of 15 Swiss albino mice weighing between 18 - 25 g (5 - 6 weeks) were

obtained from the Animal House, College of Health Sciences, Benue State University, Makurdi. They were housed in polypropylene cages at the side laboratory of the Department of Physiology, College of Health Sciences and divided into three groups of 5 animals each. The control group was fed normal chow and water *ad libidum* for a period of 12 weeks. The second group was fed HFD and water *ad libidum* for a period of 12 weeks too. The third group was fed HFD and Omega-3 (200 mg/kg) plus CBD (10 mg/kg).

2.2. High Fat Diet (HFD) Composition

The HFD was home made as described by Julia *et al.* (2018) with slight modification replacing lard with tallow [13]. Base feed, tallow and soya oil were used at inclusion rates of 60%, 25% and 15% respectively. Total energy of final feed: was 5340 kcal/kg and percentage fat in the diet was 68.7%.

2.3. Specimen Collection and Preparation

After 12 weeks the animals were sacrificed after anaesthesizing with choloroform in an enclosed chamber. The intestinal (caecal) specimen were removed and stored in sterile bottles for culture. A selective medium (salmonella-shigella agar) was used to isolate the enteric bacteria and faecal specimen. 5.7 g of the medium was dissolved in 100 ml of distilled water and heated without boiling. This was allowed to cool to 50˚C - 55˚C then mixed and dispensed aseptically into petri dishes and allowed to dry. The intestinal contents were inoculated to get a discreet colony. The colonies were sub-cultured after 24 hours in a separate petri dish in order to obtain pure culture for another 24 hours. A smear of the culture of the test organisms were prepared on a clean glass slide and heat fixed. It was stained with crystal violet solution for 60 seconds and washed with running water. Then flooded with iodine solution for 30 seconds and later decolourized by adding alcohol in drop wise manner. This was then counter stained with safranin for 2 minutes, rinsed with water again and air dried. The slides were then examined under oil immersion objective (×100). Gram negative bacteria appeared red while gram negative appeared purple [14].

2.4. Indole Test

The Indole test was carried out according to Cheesbrough (2006) [15]. The test organism were inoculated in a binjou bottle containing 3 ml of sterile peptone water. It was then incubated at 35˚C - 37˚C for 48 hours. Presence of indole was tested by adding 0.5 ml of Kovac's reagent. A surface reddish colouration within 10 minutes is a positive result.

2.5. Triple Sugar Iron Test

The triple sugar agar is a differential medium that contains sucrose, lactose, glucose, ferrous sulfate and phenol red. If an organism ferments all three sugars the medium turns yellow. If it ferments only dextrose it turns red around that area.

The inoculum was streaked on the agar slant and stabbed using sterile wire loop and incubated at 37˚C for 24 hours. Evidence of sugar fermentation and hydrogen sulphide productions were noted. The latter will give a blackening along the slant bottom junction. Fermenters like *E. coli* will ferment all the sugars. Salmonella typhimurium will ferment glucose and reduce sulphur [16].

Total viable count of bacteria colonies were done and expressed in colony forming units per milliliter (cfu/ml).

2.6. Y-Maze

Y maze is a tool used to assess working and spatial memory in experimental animals. It is based on the innate preference of an animal for novelty. A mouse with intact working memory and functional prefrontal cortex will remember the arms previously visited and show a tendency to enter a previously unentered arm. An intact spatial reference memory and functional hippocampus, can also be tested by the Y-maze [17] [18] [19]. The maze was constructed from wooden material with the three identical arms at an angle of 120˚ to each other. On commencement of the experiment one of the arms was closed with a wooden shutter and the mouse placed in the centre of the maze to explore the two open arms for a period of 5 minutes. The animal was then given a rest interval of 30 mins before the second trial; this time with all arms opened, the animal explores all three arms for another period of 5 minutes. In between trials the maze was wiped with 70% ethyl alcohol to obliterate all olfactory cues. The maximum spontaneous alternations (*i.e.* total number of arms entered minus 2) and the actual alternations (*i.e.* either ABC, ACB, BAC, BCA, CAB OR CBA) were measured. Percentage alternations were then calculated as:

$$\text{percentage alternations} = \frac{\text{actual alternations}}{\text{maximum alternations}} \times 100$$

The percentage alternation is directly proportional to memory index.

2.7. Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. The differences between groups were analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test using the SPSS statistical tool version 20. Values of $p < 0.05$ were considered significant.

3. Discussion

This research demonstrated that HFD promoted gut *E. coli* growth significantly; which was ameliorated by CBD and omega 3 (**Figure 1**). HFD was also found to suppress learning and visuospatial memory on the Y-maze experiment compared to the control group (**Figure 2**). CBD and Omega 3 (both potent antioxidant and anti-inflammatory agents) significantly improved memory impairment caused by the HFD. These findings show that HFD causes memory impairment possibly by induction of neuroinflammation and oxidative stress which has been corroborated

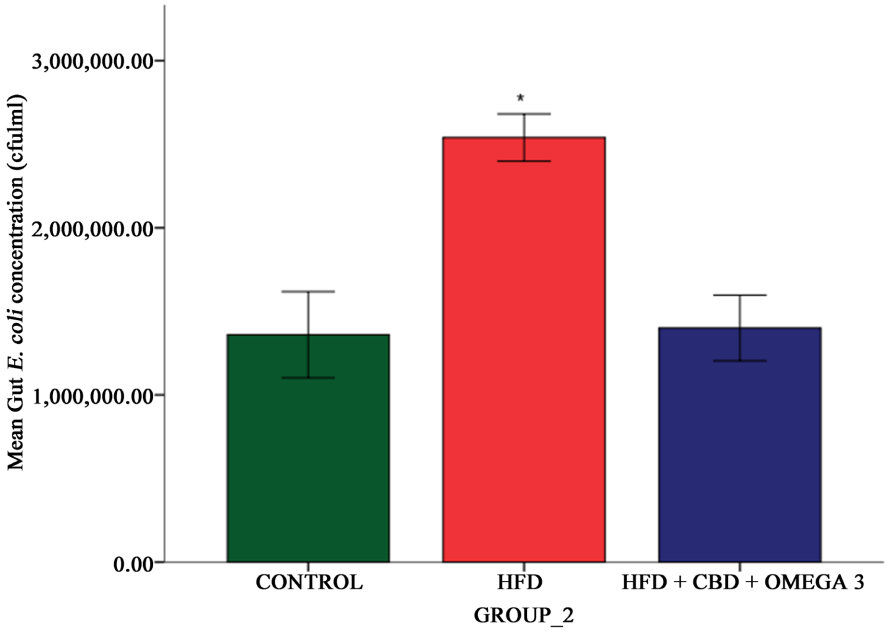

Figure 1. HFD group had a significantly higher gut *E. coli* concentration compared to the other groups. *indicates statistical significance ($p < 0.05$).

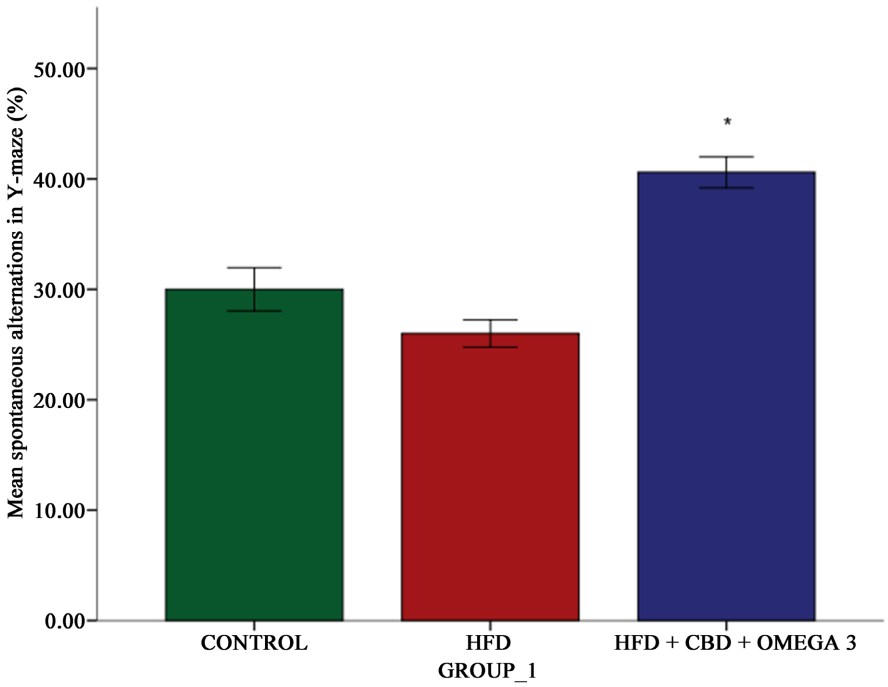

Figure 2. HFD + CBD + 0 mega 3 group had a statistically significant percentage spontaneous alternation in Y-maze compared to HFD and control groups. *indicates statistical significance ($p < 0.05$).

by some literatures [20] [21] [22] [23]. CBD and Omega 3 have individually been used in learning and memory experiments and have been known to be effective in some cases [24] [25] [26]. *E. coli* overgrowth in the gut has been implicated in the aetiology of dementia either by direct production of amyloid plaques or by

precipitating amyloid precursor proteins [27]. It is also found to be involved in the induction of gut inflammation via immune hyperstimulation allowing for toxins to reach the brain via the GBA [28] [29]. This also implies that HFD has an indirect adverse effect on learning and memory via the deposition of amyloid plaques in the brain by the *E. coli* overgrowth pathway. This experiment has demonstrated for the first time that the combination of CBD and Omega 3 reduced the neurotoxicity of HFD and also ameliorated the memory impairment it causes.

Similarly, HFD increased the anxiety levels in this animals as seen in the significantly shorter time spent in the open arm compared to the other groups (**Figure 3**). Anxiety is demonstrated experimentally using the EPM by observing the light aversion response and increased preference for the closed darker arm of the maze by the anxious animals. The research of Dutheil *et al.* (2016) corroborated our findings by demonstrating that high fat diet induced anhedonia and heightened levels of anxiety in rats [30]. CBD and omega 3 combinations significantly reduced the anxiety levels in the HFD-fed mice in our experiment. Omega 3 has been shown to maintain metabolic balance and upregulate carnitin-palmitoyl-transferase 1 gene and significantly ameliorated anxiety in ovariectomized rats [31]. Studies have also described CBD as a potential anxiolytic agent due to its possible actions on the CB1, 5HT and TRPV receptors. So, synergistically CBD and omega 3 possess a more potent anxiolytic action which would be preferred to conventional anti anxiety medications.

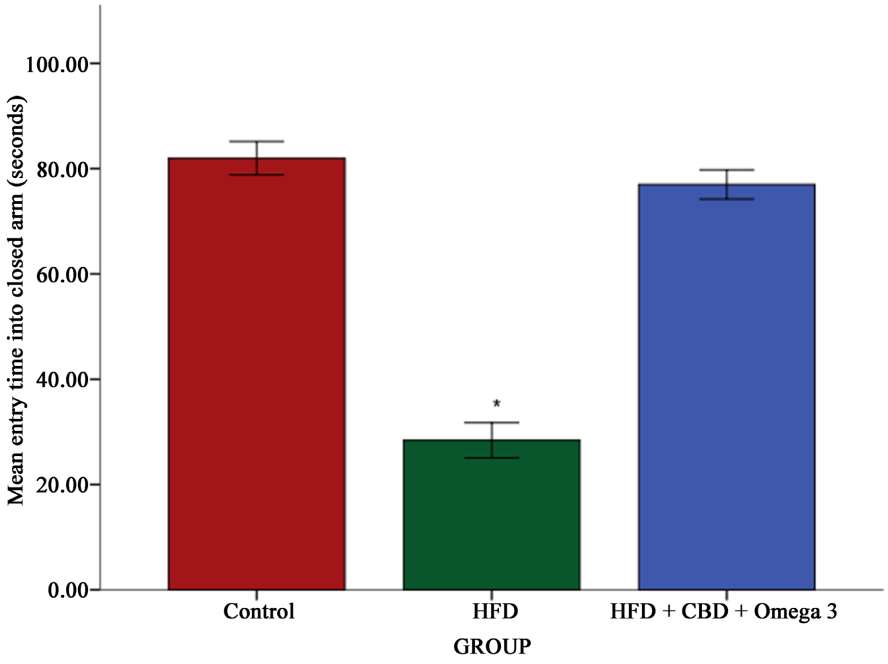

Figure 3. HFD significantly shortened the time in the open arm in the EPM test compared to the control and HFD + CBD + Omega 3 groups *indicates statistical significance ($p < 0.05$).

4. Conclusion

This research finding proved for the first time the synergistic effect of CBD and omega 3 in ameliorating the anxiety and memory impairment induced by HFD. Gut *E. coli* overgrowth was induced by the high fat diet a possible cause of the cognitive impairment. The neuroinflammation and oxidative stress associated with chronic HFD consumption were possibly countered by the anti-inflammatory and antioxidant action of CBD and omega 3. Also, the anxiety induced by HFD was ameliorated by this intervention probably by up-regulation of carnitin-palmitoyl-transferase and by acting on CB1, 5HT and TRVP. More research will be needed to establish molecular mechanism behind these findings.

Conflicts of Interest

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

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