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Modified Atkins Diet Delayed the Onset of Epileptogenesis, Improved Motor Coordination and Enhanced Learning Memory in Mice

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Abstract: Modified Atkins diet is a high fat, moderate protein and carbohydrate diet that has long been used to manage intractable epilepsy in developed countries. However, the use of this diet for same condition is uncommon in most low- and middle-income countries including Nigeria. In a previous research, we formulated modified Atkins diet using cheap and locally-available food materials in Nigeria. This research is thus, aimed at evaluating the effect of the formulated modified Atkins diet on seizure plasticity using the PTZ-induced kindling model of seizure. We also investigated the effect of sub-chronic use (60 days) of the diet on motor coordination using positional sense test and on visual-spatial reference and learning memory using Morris-water maze. The result revealed that the formulated modified Atkins diet significantly ($p<0.05$) increased seizure plasticity by reducing the severity of seizure as well as delaying the onset of tonic-clonic seizure and death. The diet also enhanced motor coordination, and improved learning memory, but not reference memory in mice. We therefore concluded that the formulated modified Atkins diet may be of benefit in the management of intractable/treatment-resistant epilepsy, especially in patients who are already suffering from amnesia and/or postural instability since the diet was able to offer protection against seizure-induced memory loss and motor-incoordination in mice.

Keywords: Nigerian-Made, Modified Atkins Diet, PTZ-Induced Kindling, Epileptogenesis, Epilepsy

1. Introduction

Epilepsy, a chronic neurological condition, affects more than 50 million people worldwide, with over 80% of this population residing in low- and middle-income countries [1]. Most epilepsy patients suffer from temporary loss of memory as well as falls due to motor incoordination [1, 2]. The vast majority of epilepsy patients are treated with conventional anticonvulsant agents [3], but more than 20% of these patients are unresponsive to these pharmacotherapies – these patients are said to suffer from intractable epilepsy. For more than a century now, the use of dietary therapy, particularly the classical ketogenic diet, has gained tremendous popularity in high-income countries for the management of treatment-resistant [4].

Modified Atkins diet is a variant of the classical ketogenic, and has been shown in many research findings to be equipotent to the classical ketogenic diet in the treatment of intractable epilepsy, particularly in children [4-7]. In a previous experiment, we demonstrated that modified Atkins diet can be formulated from cheap and locally available food materials in Nigeria [8]. The diet however had a proconvulsant effect in mice exposed to four different acute seizure tests. This result prompted the investigation of the effect of the diet on model of epileptogenesis, specifically, the PTZ-induced kindling model.

Previous research showed that the classical ketogenic diet caused memory impairment in mice following acute exposure of mice to it [9]. We therefore evaluated the superiority, or otherwise, of the formulated modified Atkins diet to the classical ketogenic diet in this regard.

2. Materials and Method

2.1. Materials

2.1.1. Food Materials and Drug

Previously formulated modified Atkins diet, normal rodent feed, pentylenetetrazole (*Sigma Aldrich, UK*).

2.1.2. Animals

Fourteen (14) male Swiss albino mice at P28 (Twenty-eight days old) weighing between 10 – 18g were used for the experiment. P28 mice were gotten from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Nigeria. The animals were kept in cages containing saw-dust beddings and were fed with the appropriate diet (either the normal laboratory diet or the formulated MAD) and water *ad libitum* with regular changing of beddings. They were maintained under normal light/dark circle.

The experimental protocols adopted in this study were as approved by the Ahmadu Bello University Committee on Animal Use and Care, ABUCAUC, (ABUCAUC/2020/30).

2.2. Methods

2.2.1. Investigating the Effect of MAD on Epileptogenesis Using PTZ-Induced Kindling

Protocol described by Shimada and Yamagata [10] was adopted. Fourteen (14) P21 male mice were divided into 2 groups of seven mice each. Mice in group I and II received laboratory diet and modified Atkins diet respectively for 30 days. Fifteen (15) alternate days administration of sub-convulsive dose of PTZ (35mg/kg, *i.p*) was done and mice were maintained on their respective diets throughout the injection period. After every PTZ administration, each mouse was observed for 30 minutes and the seizure behavior was scored as follows; 0 to represent normal behavior, 1 to represent no abnormality; 2 to represent immobilization, lying on belly; hind-limb myoclonus; facial myoclonus; head nodding; or forelimb; 3: tail held up stiffly, myoclonic jerks, continuous whole-body myoclonus, 4: falling down on its side, rearing, tonic seizure, 5: wild rushing and jumping, tonic-clonic seizure, falling down on its back; 6: death [10]. The time until death for mice in each group was also recorded.

2.2.2. Evaluating the Effect of MAD on Motor Coordination Using Positional Sense Tense

This test was conducted 24 hours after the kindling test. Method described by Holmes [11] was adopted. The hind limbs of the mice were slowly lowered over the edge of a table. Normally, a mouse will rapidly correct its abnormally placed limb. The time taken for the animal to lift its limbs back to a normal position was noted. A neurological deficit is indicated by the inability of an animal to rapidly correct such an abnormally positioned limb.

2.2.3. Evaluating the Effect of MAD on Memory Using Morris Water Maze

Protocol described by Qian *et al.,* [9] was adopted with slight modifications: in place of 6 daily trials, 4 daily trials were done; non-toxic blue dye was used instead of non-fatty milk to make the pool opaque. All mice were tested in the water maze 61 days after initiation of the MAD (i.e., 30 days after initiation of diet and 31 days following alternate days injection of sub-convulsive doses of PTZ). Mice remained on the diet for the whole of the testing period. A circular aluminum tank (50 cm high and 200 cm in diameter) was filled with water to a depth of 25 cm. About 200 mL of blue dye was added to make the water opaque and prevent mouse from visualizing the escape platform. Four points on the rim of the pool were designated north (N), south (S), east (E), and west (W), thereby dividing the pool into four quadrants (NE, NW, SE and SW). At 1 cm above the water surface, an 8 by 8cm platform onto which the mouse could escape was placed in the center of one of the quadrants. It was not possible to conceal the identity of the diet groups to an observer because of difference in fur appearance. To locate and escape onto the submerged platform, mice were trained for 16 trials on days 1–4 i.e., 4 trials daily. For each mouse, the quadrant in which the platform was located remained constant, but the point of immersion into the pool varied between north, east, south, and west in a somewhat random order for the 24 trials so that the mouse was not able to predict the platform location from the point at which it was placed into the pool. The time from immersing a mouse into the pool to the time of escape onto the platform was recorded for each trial, and the observer also manually recorded the route taken by each mouse to reach the platform. The mice were given a 30s rest period after mounting the platform and the next trial was started thereafter. If the platform is not found by a mouse in 60 s, then it was manually placed on the platform for a 30 seconds rest. At the start of each trial, each mouse was held facing away from the pool and dropped into it to ensure immersion. 24 hours after completion of the last latency trial, the submerged platform was removed and animals were placed in the water maze in the quadrant opposite to where the platform had previously been located. The time spent in the quadrant where the platform had been previously placed as well as the path taken were recorded. Normal animals typically spend more time in the quadrant where the platform had been previously located than in the other quadrants.

2.3. Statistical Analysis

Data analyses were conducted using SPSS version 25. Seizure scores were analyzed using non-parametric aligned-rank test. Time to death for mice in the kindling test was analyzed using non-parametric Kaplan Meier survival test. Data obtained from the positional sense test and Morris water maze were analyzed using Mann-Whitney test and mixed design analysis of variance respectively.

3. Results

3.1. Effect of MAD on Seizure Plasticity and Survival Probability

As shown in the figure 1, compared to normal diet-fed mice,

modified Atkins Diet (MAD) significantly ($p<0.05$) increased seizure threshold throughout the period of epileptogenesis induction compared. Based on Kaplan Meier survival analysis (figure 2), Modified Atkins Diet increased seizure plasticity in mice by delaying the time to tonic-clonic seizure such that at the end of the kindling test, mice treated with MAD had a survival probability of 60% while mice on LD had a 20% chance of not experiencing tonic-clonic seizure.

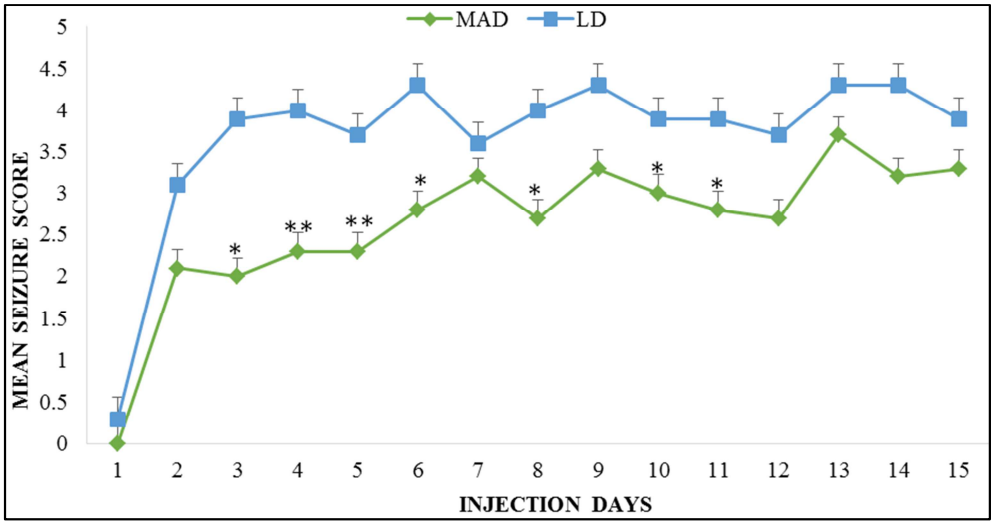

Figure 1. *Effect of MAD on Seizure Plasticity in Mice following Epileptogenesis Induction.*

Data was analyzed using Aligned-Rank analysis followed by Mann-Whitney U correction. ** = *p value* < 0.01 compared to LD; * = *p value* < 0.05 compared to LD.
Key: MAD = Modified Atkins Diet; LD = Laboratory Diet; INJ= Injection Day.

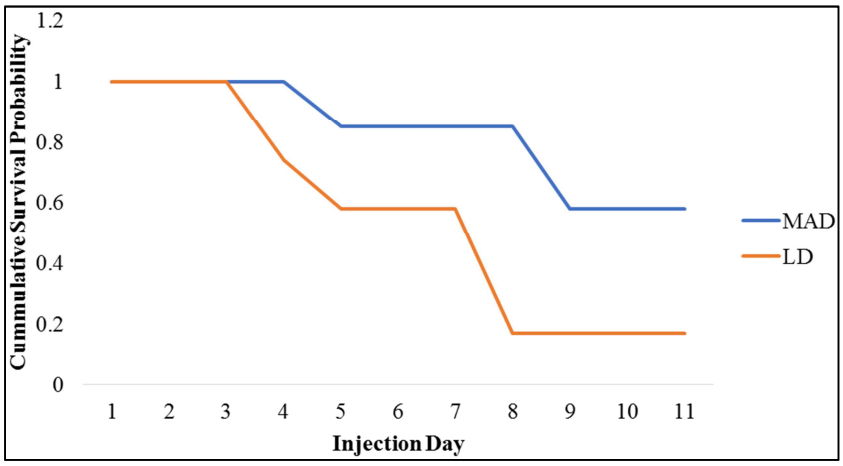

Figure 2. *Effect of MAD on Survival Probability of Mice Exposed to Sub-convulsive Dose of PTZ over 30-Day Period.*

Data was analyzed using Kaplan Meier Survival Probability for Mice Treated with Different Diet. Key: MAD = Modified Atkins Diet, LD= Laboratory Diet. *P* value is 0.049 (Log-Rank); 0.038 (Breslow); 0.041 (Tarone-Ware).

3.2. Effect of MAD on Motor Coordination

As shown in table 1, the mice that were fed with MAD were significantly ($p<0.05$) able to correct their abnormally placed hind-limbs faster (correction time of 1.25 ± 0.25) than those fed with LD (correction time of 2.57 ± 0.43).

Table 1. *Effect of Diet on Motor Coordination.*

Diet	Correction Time (seconds)
LD	2.57 ± 0.43
MAD	1.25 ± 0.25*

Correction time is in seconds and is expressed as Mean ± S.E.M. Data was analyzed using non-parametric Mann-Whitney U test. N = 7. * *p value* < 0.05.
Key: LD = Laboratory Diet; MAD= Modified Atkins Diet. N is Number of mice in each group, S.E.M = Standard Error of Mean

3.3. Effect of MAD on Visual-Spatial Memory

Modified Atkins diet enhanced learning memory and did not negatively impair reference memory. As shown in figure 3, mice that were fed with MAD displayed significant *(p<0.05)* improvement in time taken to locate the escape platform, thus increasing escape latency of the mice over time when compared to the laboratory diet-fed group in which the escape time was comparable on all test days. However, there was no significant change (*p>0.05*) in the time spent in all quadrants in both diet groups, an indication of a lack of influence on reference memory. Figure 4.

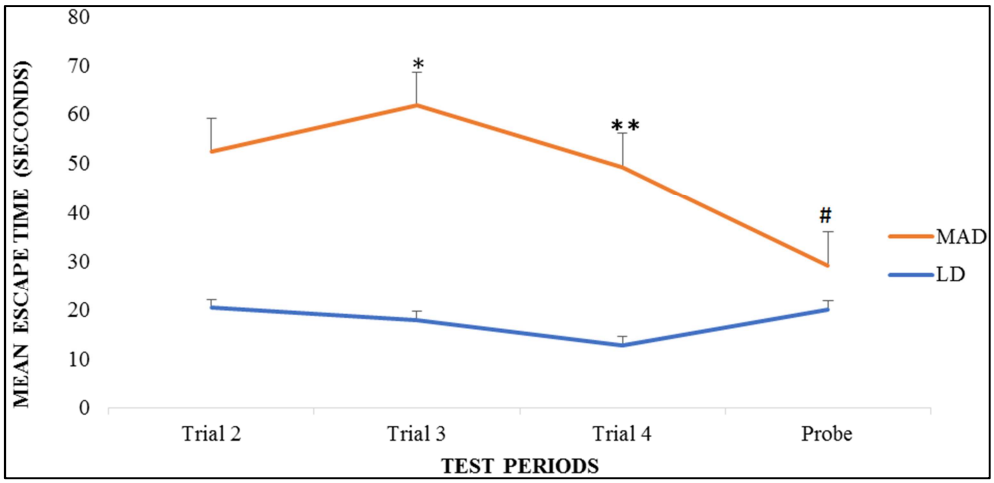

Figure 3. *Effect of MAD on Visual-Spatial Learning in Mice.*

Data was analyzed using Mixed design analysis of variance followed by Bonferroni Posthoc test. * = p value < 0.05; ** = p value < 0.01 compared to LD; # is *p value < 0.05* compared to trial 2.
Key: LD = Laboratory Diet; MAD = Modified Atkins Diet

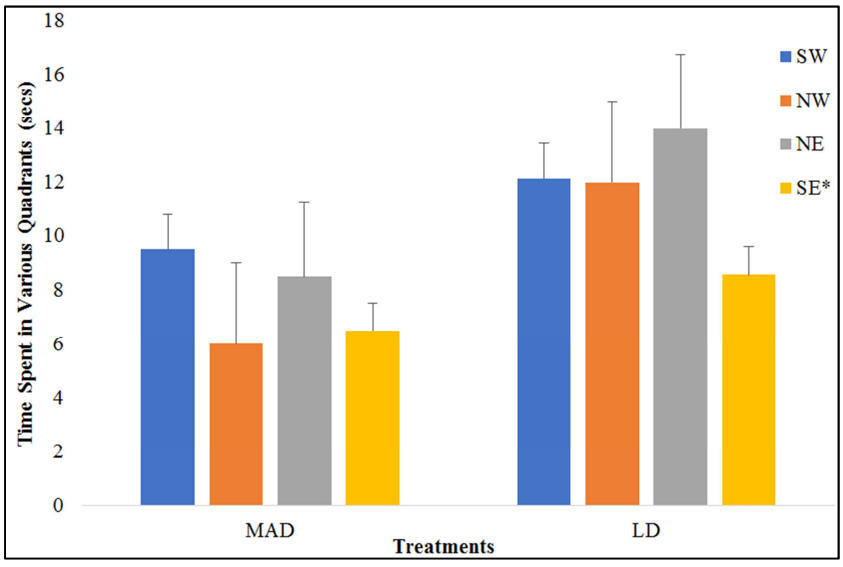

Figure 4. *Effect of MAD on Reference Memory in Mice.*

N = 7. Data was analyzed using mixed design analysis of variance and expressed as Mean ± Standard Error of Mean.
Key: LD = Laboratory Diet, MAD = Modified Atkins Diet, N = Number of mice in each group; SW=South-West quadrant, NW= North-West quadrant, NE= North-East quadrant, SE=South-East quadrant. * represent target quadrant.

4. Discussion

Chemical kindling model of seizure involves the systemic administration of sub-convulsive doses of proconvulsant agents such as pentylenetetrazole over an extended period. This process is capable of evoking a convulsive state in animals by reducing seizure threshold [10] and producing spontaneous recurrent seizure (SRS) [12] thus, makes the kindling model a robust model of epileptogenesis. Any

substance that is therefore capable of preventing the occurrence of SRS or delaying it or preventing death of animals following the induction of SRS is said to possess antiepileptogenic activity [13]. In this experiment, the formulated modified Atkins diet was able to delay the emergence of SRS as well as reduce seizure-related mortality in the mice. It can thus be said to have antiepileptogenic activity. Because the diet had a proconvulsant effect in mice exposed to acute models of seizure, it was considered unlikely to produce antiepileptogenic effect in the same animal species. This finding isn't surprising however, considering that modified Atkins diet formulated from other food materials have proven to be beneficial in the management of treatment-resistant epilepsy in clinical settings. Moreover, certain anticonvulsant agents like levetiracetam, which did not confer protection to animals in all acute models of seizure as well as some chronic models, is now one of the most effective AEDs in the treatment of epilepsy in humans [13].

Several mechanisms have been proposed to be responsible for the anticonvulsant activity of ketogenic diets. One such mechanism is mitochondrial biogenesis, which involves the generation of adenosine monophosphate (AMP) from the breakdown of adenosine triphosphate (ATP)), following its movement from neuron to synapse, in an attempt to activate neuronal K^+-channels through phosphorylation, thereby promoting hyperpolarization of activated neurons [14]. Mitochondrial biogenesis is a chronic process that is activated under increased energetic needs by a specific signaling pathway [15], this perhaps explains why the formulated diet was only able to prevent epileptogenesis, a chronic phenomenon, and not seizures induced acutely. The anticonvulsant activity of ketogenic diet has also been attributed to other chronic processes such as neurotransmitter modulation i.e., enhancement of GABA activation and or inhibition of glutamate transmission [16].

Memory deficit is a common phenomenon in patients with focal epilepsy [17], as such we evaluated the effect of sub-chronic use of the formulated diet on memory using Morris water maze. Morris water maze is used to evaluate spatial learning in rodents that depend on distal cues to navigate locations in an open swimming arena until an escape platform placed below the water surface is located. Spatial learning is determined over repeated trials and reference memory is assessed by preference of a rodent for the target quadrant when the platform is absent [18]. Here, several parameters were observed and recorded- average distance to the target site, time spent in or distance covered within the target quadrant. MAD did not negatively impact the reference memory of mice evidenced in a comparable time spent in the target quadrant between LD- and MAD-fed mice. The diet however, enhanced the mice learning memory which was reflected in a gradual improvement in escape time over the entire trial days of MAD-treated mice compared to LD-treated mice. The ability of the formulated modified Atkins diet to enhance learning memory may be linked to its effect of GABA transmission, which has been reported in several researches to play a positive role in memory formation [19-21].

Epilepsy is associated with some neurological deficits including loss of motor coordination leading to frequent falls [1]. In the simple yet valid positional sense test, mice that were fed modified Atkins diet showed enhanced motor coordination because they were able to quickly correct their abnormally placed limb. A result that is reflective of the ability of the diet to improve motor coordination.

5. Conclusion

The formulated modified Atkins diet significantly increased seizure plasticity by delaying the onset and severity of seizure, the diet also enhanced visual-spatial learning memory and improved motor coordination in mice.

Abbreviation

PTZ: Pentylenetetrazole
MAD: Modified Atkins Diet

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