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The Role of Coconut Oil on Pentylenetetrazole Induced Convulsion in Rats: An Electroencephalogram Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author MA designed the study, wrote the protocol and corrected the manuscript. Authors OO and OA performed statistical analysis, managed the analysis and literature of the study and manuscript. Author ASS was involved in administrations and treatment. Author OS prepared and provided the coconut oil. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study was carried out to investigate the role of coconut oil on pentylenetetrazole (PTZ) induced convulsant activity in Wistar rats using the laboratory model. Convulsant activity was achieved by injection of PTZ.
Study Design: The rats were divided into five groups. Group 1 served as control and received distilled water orally. Group2 was a reference group and received only PTZ. The remaining three groups (3, 4 and 5) were test groups and rats were given oral administration of coconut oil at doses of 2, 4 and 5.3 ml/kg for 21 days.
Methodology: 25 rats weighing between 125 and 200g were used. 30 minutes after the last administration of coconut oil after 21 days, a convulsive dose of PTZ was given intraperitoneally. Electroencephalogram (EEG) readings of the rats were then taken using an EEG machine and electrodes placed on the head of the rats recorded the waves produced on the scalp of the rats. The frequencies of the waves recorded were analyzed and compared for all groups.

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Result: The frequency of EEG readings produced during convulsion that was caused by the PTZ in the rats were reduced for the rats that received coconut oil. There was a significant decrease in the mean frequency of EEG of rats that received 2, 4 and 5.3 ml/kg coconut oil (which had frequencies of 13Hz, 14.6Hz and 14. 4Hz, respectively) when compared with the reference group that received only PTZ which had a mean frequency of 16Hz. Coconut oil significantly reduced the frequency produced by PTZ. The result also shows that the lower dose group had the most appreciable decline in convulsive activity returning the frequency of electroencephalogram waves recorded to 13Hz same as the control group.
Conclusion: The result suggests that coconut oil given at a moderate dose has anticonvulsant effect and will cause an increase in weight. These findings supports reports that ketogenic diet could help reduce convulsant activities and epileptic seizures.

Keywords: EEG; coconut oil; pentylenetetrazole; convulsion; anticonvulsant; ketogenic diet; epilepsy.

1. INTRODUCTION

When the normal balance between inhibition and excitation is significantly disrupted in all or part of the brain, a seizure can occur which leads to convulsion [1]. Convulsion can therefore be referred to as the uncontrolled involuntary muscular contractions, which are caused due to the paroxysmal uncontrolled discharge of impulses from neurons of brain particularly the cerebral cortex [1,2,3]. Convulsion has been well studied to be treated over the years with anticonvulsant pharmacodynamic group of drugs such as barbiturates. The GABA system is an important target for anticonvulsant drugs, since seizures may be reduced by increasing GABA synthesis, decreasing its breakdown or enhancing its effect on neurons [2,4].

Convulsion can also be treated with non-medical procedures and one of the most commonly use non medical substances is the ketogenic diet [5,6]. The ketogenic diet is a high-fat, adequate-protein, low-carbohydrate diet primarily used to treat difficult-to-control refractory epilepsy [7]. The diet forces the body to burn fats rather than carbohydrates. The carbohydrates found in food are normally converted into glucose, which is transported around the body and is also important in fuelling the brain function. However, the liver converts fat into fatty acids and ketone bodies [8]. The ketone bodies then pass into the brain and replace glucose as an energy source. An elevated level of ketone bodies in the blood, a state known as ketosis, leads to a reduction in the frequency of convulsion and epileptic seizures [9,10].

Coconut oil is a major ketogenic diet and is of special interest because it possesses healing properties far beyond any of the other dietary oil and also provides a wide range of health benefits [5,6,11]. Coconut oil is different from most dietary oils because of the presence of basic building blocks or fatty acids making up the oil [11]. Coconut oil is composed predominantly of a special group of fat molecules known as medium chain fatty acids (MCFA). Coconut oil contains 92% saturated fatty acids. The saturated fatty acids contain mostly medium chain triglycerides which are made up of lauric acid, capric acid, caprylic acid, myristic acid and palmitic acid [12]. The ketogenic diet results in adaptive changes to brain energy metabolism that increase the energy reserves. This may help the neurons to remain stable in the presence of increased energy demand during a seizure, and may confer a neuroprotective effect. The ketogenic diet has also been found to have antiepileptogenic properties in rats [11,13].

EEG studies have been used to report the presence of seizures and convulsion in the brain and studies have reported a number of techniques for EEG readings, examples include the use of network techniques (as well as related statistical clustering techniques) for EEG interpretation [14-16]. Drewes et al. [17] used spectral (fourier) analysis followed by unsupervised clustering in study of EEG of sleep in fibromyalgia. There has also being the use of analyzed amplitude and frequency in interpretation of EEG readings as well as the use of morphological arrangement (symmetrical or asymmetrical) to report the type of wave produced in the cerebral cortex due to drug action or seizures. [15,16,17].

The study is aimed at showing the role of coconut oil which has ketogenic properties in the PTZ induced convulsing rats using an EEG.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Preparation of coconut oil

The white coconut meat (100 g) is grated and then ground using a blender. One liter of water is boiled and poured into the grounded coconut meat and then sieved with cheese cloth and left for 24 hours, the white layer is then scraped into a pot and placed on fire, it is left to boil for approximately 1hr 30 minutes till the oil comes up and it is then collected using a cooking spoon with a wooden handle. The oil is administered at different doses orally to the rats daily for 21 days.

2.1.2 Instrumentation

1. Oral administration tube
2. EEG electrode Cap - EEG reading
3. Complex wave Chart - Recording EEG readings
4. EEG machine / Physiographic recorder

2.1.3 Animals

Male Wistar rats weighing 125 - 200 g were employed. They had free access to food and water except at the time of the experiments. The animals used were acclimatized for two weeks before the study. The rats received normal rat chores throughout the course of study. The ethical approval on the research protocol was received from the Ethics review committee of the University a branch of the National Universities Ethics committee on research protocols, and the research was adequately carried out within the ethics review guidelines for research using animals.

2.2 Experimental Design

Group 1 served as control group to which they received distilled water orally while group 2 was treated but with only PTZ, intraperitoneally with a dose of 60 mg/kg (0.24ml) of body weight and did not recieve coconut oil. The other 3 Groups were given coconut oil orally at increasing quantities of 2 ml/kg bodyweight for Group 3, 4 ml/kg bodyweight for Group 4, 5.3 ml/kg body weight for Group 5, daily for 21 days after which all groups except Group 1 were induced with convulsion by administering a dose of 60 mg/kg of PTZ.

The effect of the coconut oil was evaluated based on the ability of the oil to prolong the duration of the latent period (i.e. the time taken for the onset of clonuses with a loss of the righting reflex or tonic hind limb extension, whichever appeared first after the administration of PTZ) and its ability to decrease mortality. The EEG study was done immediately after administration of PTZ to study the waves produced and the time involved in convulsing and non - convulsing rats for comparison [18].

An EEG with bipolar electrodes on an EEG cap was used to record EEG reading connected to a Student's physiograph. A BD Instrumentation Labotech, 3 Channel Physiograph with Biopotential Coupler was used with sensitivity - 1mV/cm, Speed - 10mm/s. The electrodes from the instrument were placed over the scraped and unopened scalp of rats. The bipolar electrodes were used and their terminals were placed on different parts of the brain. The nasion, an indentation between the forehead and the nose, the inion, a ridge that can be felt at the midline of the back of the skull, over the occipital area, and the preauricular points are defined as the indentations just above the cartilage that covers the external ear openings as the reference electrode [16,19].

Complex wave forms were recorded on a graph paper with the aid of the styloink on the physiograph for 5 minutes during which stable (with little or no movements from the rat) and unstable (movement by rat due to environmental influence) of the rats were marked on the chart. The EEG readings taken are then analysed and reported in amplitude and frequency of the waveforms.

2.3 Statistical Analysis

Statistical analysis of results was carried out using the Student's t-test. P values were set at $P<0.05$. The frequencies (number of waves per second) and amplitudes (height of the waves) of complex wave forms when the rat was stable and unstable were separately recorded and the means for each group compared statistically for significant difference. The sets of results were reported with their levels of significance accordingly.

3. RESULTS

The results below show the mean ±standard error (S.E) of mean weight of rats before and after treatment with coconut oil for all groups Table 1, while Table 2 shows changes in frequency and amplitude due to administration of PTZ after coconut oil has been given for 21 days. Frequency in waves per second (Hertz) and the amplitude in millimeters. PTZ and coconut oil were administered according to body weight.

EEG readings taken were recorded with respect to the aptitude and frequency. Due to activities taking place during the experiment the EEG readings of rats were taken when stable (no external disturbance) and unstable (with movements due to environmental influence).

The results show changes in weight due to coconut oil and in values for the amplitude and frequency due to PTZ administration after coconut oil was given. Fig. 1 shows the changes in amplitude and Fig. 2 shows the changes in frequency of the EEG readings recorded and how coconut oil affects.

Table 1. Mean weight of rats in groups 1 to 5

Groups n=5	Coconut Oil (ml)	PTZ (ml)	Weight (g)	
			Before	After
1	0	0	149.6±2.0	151.4±3.42
2	0	14.66±1.32	146.6±5.9	145.8±10.41
3	0.30±0.04	16.80±1.64	132.0±2.0	168.0±7.5$^{*\#+}$
4	0.66±0.05	16.12±3.37	147.8±8.2	161.2±15.1$^{*\#+}$
5	0.88±0.08	23.80±2.68	163.6±8.5	238.0±12.0$^{*\#+}$

** indicate values that are significantly different when compared with the control group (1) at (P<0.05), $^{+}$ indicate values that are significantly different when compared with the PTZ induced group (2) and $^{\#}$ indicate values that are significantly different when before administration is compared with after within same group at (P<0.05), All values are Mean ± S.E. M for each group. Weight in (g)*

Table 2. Mean frequency and amplitude of rats in groups 1 to 5

Groups n=5	Amplitude (mm)		Frequency (Hertz)	
	Stable	Unstable	Stable	Unstable
1	1.3±0.1	4.7±0.3	13.0±0	5.6±1.2
2	1.42±0.1	5.3±0.5	16.0±0*	5.0±1.1
3	1.24±0.1	4.6±0.5	13.0±0.5^{+}	5.2±0.4
4	1.06±0.04	2.7±0.2^{*+}	14.6±0.3^{*+}	5.2±0.4
5	1.46±0.1	4.5±0.4	14.4±0.3^{*+}	3.6±0.3

** indicate values that are significant different when compared with the control group (1) at (P<0.05), $^{+}$ indicate values that are significant different when compared with the PTZ induced group (2) at (P<0.05), All values are Mean ± S.E. M for each group. Frequency in Hz and Amplitude in mm.*

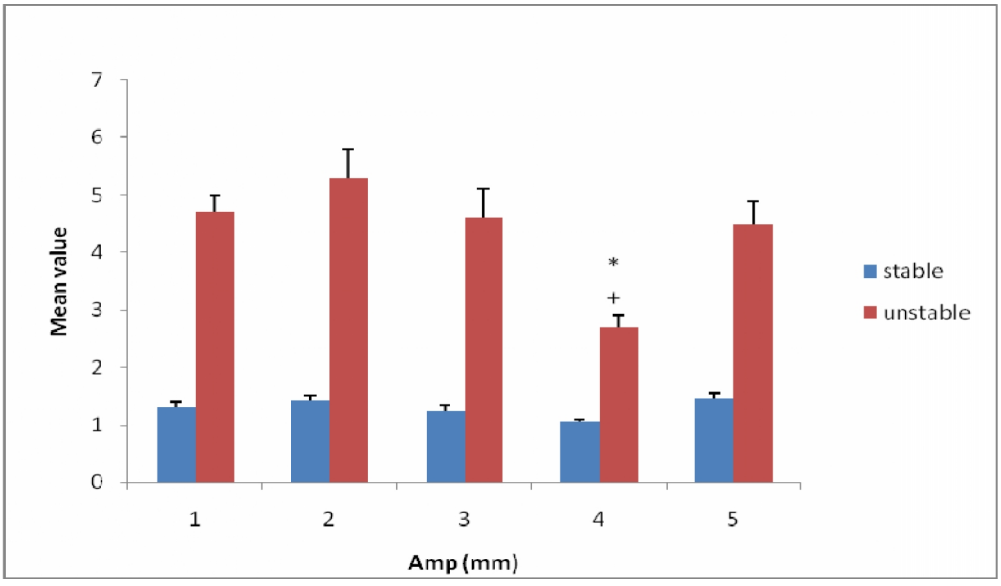

Fig. 1. Showing the Mean Amplitude (mm) of rats in groups 1 to 5

** indicate values that are significantly different when compared with the control group at (P<0.05), $^{+}$ indicate values that are significant different when compared with the induced group at (P<0.05), All values are Mean ± S.E.M for each group. Amplitude in mm.*

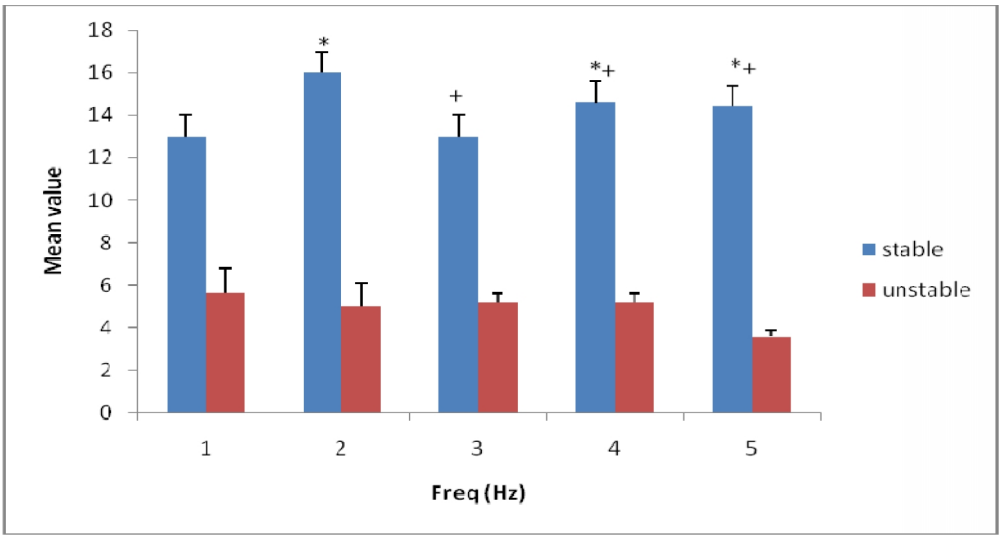

Fig. 2. Showing the Mean frequency (Hz) of rats in groups 1 to 5

** indicate values that are significant different when compared with the control group at (P<0.05), + indicate values that are significant different when compared with the PTZ induced group at (P<0.05), All values are Mean ± S.E. M for each group. Frequency in Hz.*

4. DISCUSSION

The results of the study show that rats that received coconut oil had significant increase (*P*<0.05) in body weight over the course of the experiment when their weights are compared with control and PTZ induced groups 1 and 2 that did not receive coconut oil. There was also significant increase in weight when their weights before coconut oil was administered are compared with the weights after the 21 days of coconut oil administration. This could be due to the fatty composition of coconut oil and its other components [8,9,13]. The increase in weight for the high dose group that received 5.3 ml/kg coconut oil increased from a mean of 163.6g before administration to a mean weight of 238g after administration Table 1.

The results of the EEG studies on Table 2 and Fig. 1 shows that the amplitude had no significant difference during the stable state though in the unstable state the medium coconut oil group (4) was significantly reduced compared with the control and the reference groups (1 and 2) with mean amplitude of 2.7±0.2 (*P*<0.05).

For the frequency, Table 2 and Fig. 2 show that during the stable state there was a significant increase (*P*<0.05) in the reference group, the medium group and the high dose coconut oil group (2, 4 and 5) this was due to the PTZ induced. Comparing the coconut oil groups (3, 4 and 5) with the (PTZ only) reference group (2) which had a mean frequency of 16.0±0 there was significant reduction in the mean frequency produced which were 13.0±0.5, 14.6±0.3 and 14.4±0.3 respectively (*P*<0.05). The effect of PTZ induced convulsion reduced the frequency of EEG waves produced and the low coconut oil group (3) was reduced to 13.0±0.5 same with control group (1) with a mean frequency of 13Hz in the stable state. In the unstable state there was no significant reduction in all groups.

Results shown in Figs. 3, 4, 5 and 6 are sample charts showing complex wave forms from electrodes of the EEG machine. They represent samples of waves analyzed for the data

presented in Figs. 1 and 2 and Table 2. The range of the alpha waves is between 7.5 and 13 waves per second the major rhythm seen in normal relaxed adults as seen in control and low coconut oil groups [15,16]. It also shows that the medium and high doses of the coconut oil though did not return the PTZ induced convulsion back to the frequency of the control as seen with the low dose significantly reduced PTZ effect which produced beta waves (wave seen during convulsion) as in the reference group (16Hz).

EEG Traces

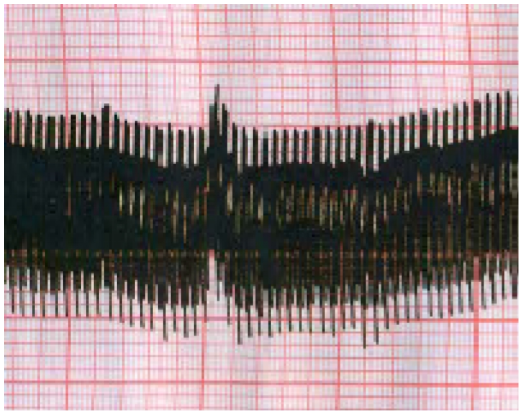

Fig. 3. Stable control group

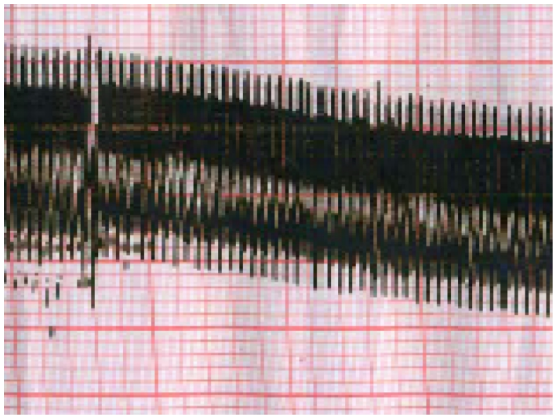

Fig. 4. Stable with PTZ only

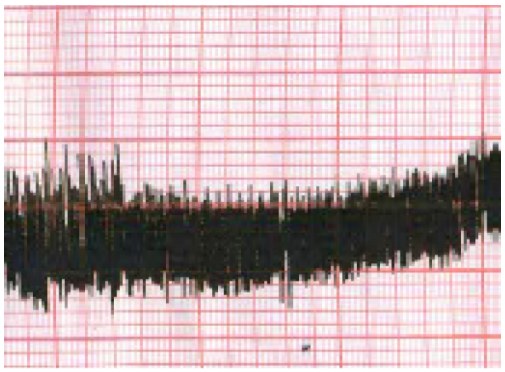

Fig. 5. Unstable with coconut oil + PTZ

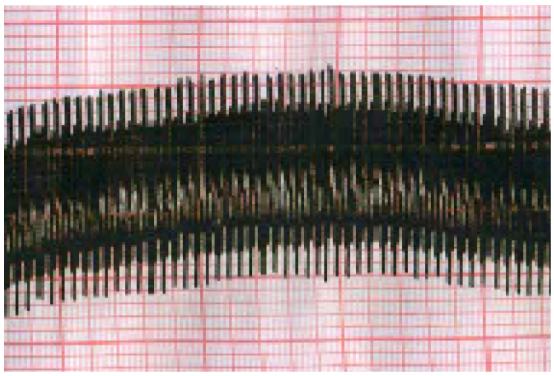

Fig. 6. Stable with Coconut oil + PTZ

5. CONCLUSION

This study showed that coconut oil administered to rats for 21 days significantly reduced PTZ effect as reflected in the frequency and amplitude produced in the EEG readings taken and suggesting it may have anticonvulsant property [3]. As can be seen with the EEG results presented the low coconut oil group had no significant difference with the control group indicative of the effect of coconut oil. This suggests that coconut oil's effect may be dose depended as the lower dose of coconut oil had a better effect. Furthermore study results show that coconut oil caused an appreciable increase in body weight after treatment when

compared with their weights at the onset of the experiment and the weights of the groups that did not receive coconut oil.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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