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Research Article

Evaluation of the Role of Ellagic Acid on Spatial Memory Activity and Oxidative Responses in Pentylenetetrazole Chronic Epileptic Rat Model

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ABSTRACT

Background: Epilepsy is a chronic neurological condition characterised by recurrent unprovoked seizures which are due to abnormal, excessive or synchronous neuronal activity in the brain. An imbalance between excitatory and inhibitory neurotransmission of the brain, as a result of a decrease in GABAergic and/or an increase in glutamatergic transmission, plays a role in the generation of epilepsy. This study assessed the effect of ellagic acid in pentyleneterazole-kindled rats and its role in spatial memory. **Methods**: Sixty (60) male wistar rats weighing between 200 – 300g were randomly divided into six groups with 10 rats each. Groups I – V administered with 35mg/kg pentyleneterazole, while group VI received distilled water subcutaneously (s.c) on alternate days for forty days. One hour before pentyleneterazole administration, group II, III and IV received 15, 30 and 60 mg/kg (i.p) ellagic acid respectively and group V received 30mg/kg Phenobarbital (i.p) and were observed for seizure activity 30 minutes after the pentyleneterazole injection. When kindling was achieved, elevated plus maze and Y-maze tests for spatial memory were done. After which the rats were anaesthetised and brain tissue were removed. The brain tissues were homogenised, centrifuged and the supernatant assayed for oxidative stress biomarker, malondialdehyde, antioxidant enzymes, superoxide dismutase and catalase activities. **Results**: The result showed significant ($p < 0.05$) increase in glutamate and malondialdehyde in group I when compared to other groups but reduction in Y-aminobutyric acid. There was increase in learning and retention ability in group IV as compared to the group I control in the elevated plus maze test, and higher Y-Maze % mean score when compared to group I control. **Conclusion**: Ellagic acid improves spatial learning and memory, protects against seizures through mitigating oxidative stress by increasing antioxidants capacities and lowering glutamate concentration.

INTRODUCTION

Epilepsy is a chronic neurological condition characterised by recurrent unprovoked seizures which are due to abnormal, excessive or synchronous neuronal activity in the brain (Fisher *et al*., 2014). Epileptic seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking (WHO, 2012). In epilepsy, seizures tend to recur, and have no immediate underlying cause (Cheng and Lowenstein, 2003). Most epilepsy has unknown causes, although it could be as a result of brain injury, stroke, brain tumour, drug and alcohol misuse. An imbalance between excitatory and inhibitory neurotransmission of the brain, as a result of a decrease in GABAergic and/or an increase in glutamatergic transmission, plays a role in the generation of epilepsy (Pena and Tapia, 2000). Genetic mutations are also linked to a small proportion of the disease. Antiepileptic drugs (AEDs), exerts their anticonvulsant activity by potentiating inhibition of excitatory neurotransmission by many mechanisms (Khongsombat *et al*., 2008). Kindling is a chronic model of epileptogenesis which is a repeated chemical or electrical stimulation of the brain that initially does not induce seizures but lowers the seizure threshold which eventually leads to the occurrence of seizures (Morimoto *et al*., 2004). Among the chemicals used to kindle animals, Pentylenetetrazole (PTZ) is widely used for its convenience (Ito *et al*., 1977).

Cognitive impairments with abnormal behaviour have been reported in more than half of epileptics due to seizures that commonly affect attention, memory, mental speed and language, as well as executive and social functions (Orrin, 2004). Apart from epilepsy itself causing cognitive impairment, antiepileptic drugs have been shown to induce cognitive impairment in patients with epilepsy (Pitkanen and Sutula, 2002). PTZ kindling provides a useful model for post seizure dysfunction, serving as a screen for potential treatments for the cognitive and emotional deficits that are observed in human epilepsy (Mortazavi, *et al*., 2005).

A relationship between free radical and scavenger enzymes in epilepsy has been established, and reactive oxygen species (ROS) have been implicated in seizure-induced neurodegeneration (Militão *et al*., 2010). Current studies have suggested that antioxidant compounds protect mitochondria and reduce oxidative stress-related events, thereby, affording some level of neuroprotection against the neurotoxicity of seizures (Santos *et al*., 2009; Militão *et al*., 2010). Lipid peroxidation and ROS production are accompanied by reduction in antioxidant defenses that aggravate the condition. Exogenous antioxidant supplementation can reverse this scenario (Gupta *et al*., 2010). Ellagic acid (EA), a flavonoid found in numerous fruits and vegetables, has been receiving large attention because of its biological properties, such as antioxidant, free radical scavenging, chemo-preventive and anti-apoptotic actions (Girish *et al*., 2014). Experimental evidences have clearly demonstrated that flavonoids exert antiepileptic activity by modulating the GABAA-Cl-channel complex, as they are structurally similar to benzodiazepines (Choudhary *et al*., 2011). Thus, flavonoids may have a modulating role in the treatment of neurodegenerative diseases due to their phenolic nature, since they can disrupt cellular oxidative processes in the central nervous system (Diniz *et al*., 2015).

The rationale behind this study therefore is the need to find solutions to seizures and also freedom from adverse side effects of commonly used AEDs which include memory impairment by utilizing natural agents which are part of the composition of some vegetables and fruits around us.

METHODS

Experimental animals and protocol

A total of sixty (60) male Wistar rats were used for this study. Male rats were used due to the variable nature of female data caused by hormonal fluctuations which has been associated with the female reproductive cycle. The rats were purchased and kept in plastic cages at the animal house, Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. They had access to water and feed *ad libitum*. All animals were acclimatised to the environment for more than two weeks prior to the commencement of the experiments. Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2016/042). And the experiments were conducted in accordance with the laboratory care policy on animal research of the Ahmadu Bello University, Zaria.

Experimental protocol was carried as described by Mehla *et al*. (2010) and Zhou *et al*. (2014) and rats were divided into six groups of 10 each. Phenobarbital (PB) was used as standard drug for negative control because of its dual effects as antiepileptic and anxiolytic.

Group 1: 35mg/kg PTZ
Group 2: 15mg/kg EA + 35mg/kg PTZ
Group 3: 30mg/kg EA + 35mg/kg PTZ
Group 4: 60mg/kg EA + 35mg/kg PTZ
Group 5: 30mg/kg PB + 35mg/kg PTZ
Group 6: Distilled water

Pentylenetetrazole-induced seizures

Pentylenetetrazole was dissolved in distilled water and injected subcutaneously (s.c) in a sub-convulsive dose of 35 mg/kg on every alternate day (48 ± 1 h) up to day 40 when seizure stage 4 or 5 on two consecutive trials was achieved (Mandhane *et al*., 2007; Mehla *et al*., 2010). The rats were pre-treated with EA and standard drug, PB, 1 hour before PTZ administration and observed for 30 minutes after each PTZ injection for convulsive behaviour (Mehla *et al*., 2010). Seizure activity was evaluated using the following scale (Racine, 1972):

Stage 0: No response
Stage 1: Hyperactivity, vibrissae twitching
Stage 2: Head nodding, head clonus, myoclonic jerk, convulsive wave throughout the body.
Stage 3: Unilateral forelimb clonus, myoclonic jerks, upright position with bilateral forelimb clonus.
Stage 4: Bilateral forelimb clonus with or without rearing, tonic-clonic seizure, turning over unto one side.
Stage 5: Generalised tonic-clonic seizures (GTCS) characterised by rearing and falling, turning over unto the back.

Animals were considered kindled after exhibiting stages 4 or 5 of seizures on two consecutive trials. And the following tests for spatial memory were conducted:

Elevated Plus Maze Test for Spatial Memory

Memory in rats was assessed using elevated plus maze for memory as described by Sharma and Kulkarni (1992). The maze consists of two closed and two open arms (50 × 10 cm) crossed at an open centre (5 × 5 cm) in plus shape. The closed arms were surrounded by high wall (40 cm by 40cm) and the whole apparatus was raised to a height of 50cm above the floor. In the 1st trial, the time the animal took to enter a closed arm with all four limbs when placed at the end of one arm facing away from central platform was recorded as the initial transfer latency. A 60s cut-off was set. The rat was allowed to move freely in the maze regardless of open and closed arms for another 10s. The maze was then cleaned with a solution of 70% ethyl alcohol and allowed to dry between tests to avoid any olfactory cue. Twenty-four hours later, retention transfer latency test was performed in the same way as in the acquisition trial. When a rat did not enter the enclosed arm within 60s on 2nd trial, the transfer latency was assigned 60s.

Y-Maze Model (Spatial Working Memory Test)

The Spontaneous alternation version of Y-maze testing as described by Hughes (2004) was employed for this study. The Y-maze is composed of three equally spaced arms (at 120°, arm's length 50 cm, width 10 cm, and wall height 20 cm). This test is based on the innate preference of animals to explore an arm that has not been previously explored. In this version, each rat was placed in the Y-maze for 5 min and the number of arms entered as well as the sequence of entries was recorded and a score was calculated to determine alternation rate. An alternation is defined as entry into all three arms consecutively (Hughes, 2004), for instance if the animal makes the following arm entries; A,C,C.A,B,C,A,C,B,A,B,C,A in this example, the animal made 13 arm entries 7 of which are correct alternations. The number of maximum spontaneous alternations is then the total number of arms entered minus two, and the percentage alternation is calculated as (actual alternations /maximum alternations x 100). A high alternation rate is indicative of sustained spatial working memory as the animals must remember which arm was entered last to not re-enter it (Hughes, 2004).

Animal sacrifice and tissue processing

At the end of the behavioural studies, the rats were fasted overnight and subsequently anaesthetised with combination of ketamine (75 mg/kg) and diazepam (5 mg/kg). They were dissected and the brain removed and washed in phosphate buffer solution (pH 7.4). Some brain tissues were homogenized with phosphate buffer solution, centrifuged at 2000 rpm for approximately 20 min. The supernatants were carefully collected and used for the ELISA kit assays.

BIOCHEMICAL ASSAY

The assessment of the biomarkers malondialdehyde (MDA), antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and gamma aminobutyric acid (GABA) and Glutamate were done using their specific commercially sourced rat ELISA kits.

Assessment of Oxidative Stress Biomarker

Samples of the brain tissue were grinded in a cold glass mortar and homogenized (1 g of tissue/9ml) in 100 Mm phosphate buffer (pH 7.4). MDA which is a measure of lipid peroxidation was measured using MDA assay kit from Northwest Life Sciences Specialities, product NWK-MDA01, Vancouver WA, Specificity: MDA sensitivity: 0.08 µM). The level of thiobarbituric-acid reactive substance was determined based on the principle of its reaction with MDA to form MDA-TBA2 adduct that absorbs strongly at 532 nm (Janero, 1990)

Assessment of antioxidant enzymes

Superoxide dismutase activity: Brain samples were grinded in a cold glass mortar and homogenized (1 g of tissue/9 ml) in 100 mM phosphate buffer (7.4). Activity of SOD was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/Ml). The auto oxidation reaction was started by addition of freshly prepared pyrogallol solution to Tris-Hcl buffer at pH 8.5. The 50% inhibition of haematoxylin by SOD was measured by spectrophotometer at 420 nm (Martin *et al*., 1987).

Catalase activity: Catalase activity in brain homogenate was determined according to method of Beer and Sizer (1952), which is based on the principle of catalase consumption of H_2O_2 substrate at 240 nm. CAT activity was assessed using NWLSS CAT activity assay kit (Product NWK-CAT01, specificity: Catalase, Sensitivity 6.0 U Catalase/mL).

Determination of glutamate concentration by ELISA technique

Rat Glutamate ELISA Kit (GA-E3963RT) was used to assay the glutamate in the sample of rat brain. This kit used ELISA based on biotin double antibody sandwich technology to assay Rat Glutamate. Glutamate was added to wells (containing 40 µl samples) that are pre-coated with glutamate monoclonal antibody (10 µl) and then incubated at 37 ^{o}C for 60 minutes. After incubation, anti-glutamate antibodies labelled with biotin were added to unite with Streptavidin-HRP (50 µl), which formed the immune complex. The plate was washed five times and Chromogen solution A and B were added. It was incubated for 10 min at 37 ^{o}C away from light in a dark chamber for colour development. The solution turned to blue. 50 µl stop solution was added to each well to stop the reaction and colour changed immediately to yellow. The absorbance of each well was measured one by one under 450nm wavelength within 10 minutes of stopping the reaction. Glutamate concentration is determined by comparing the 450 nm absorbance of sample wells to the absorbance of a known standard.

Determination of gamma aminoutyric acid concentration

Rat GABA ELISA Kit (GenAsia assay kit; GA-E0111RT, specificity: Rat GABA, Sensitivity: 2.9nmol/L) was used to assay the GABA in the sample of rat brain. This kit used ELISA based on biotin double antibody sandwich technology to assay Rat GABA. GABA was added to wells (containing 40µl sample) that are pre-coated with GABA monoclonal antibody (10 µl) and then incubated at 37 ^{o}C for 60 min. After incubation, anti-GABA antibodies labelled with biotin were added to unite with Streptavidin-HRP (50 µl), which formed the immune complex. The plate was washed five times and Chromogen solution A and B were added. It was incubated for 10 min at 37 ^{o}C away from light in a dark chamber for colour development. The solution turned to blue. 50 µl stop solution was added to each well to stop the reaction and colour changed immediately to yellow. The absorbance of each well was measured one by one under 450 nm wavelengths within 10 min of stopping the reaction. The concentration of corresponding sample was determined according to the absorbance value of the sample.

DATA ANALYSIS

Data were analysed by One Way Analysis of Variance (ANOVA) followed by Tukey post hoc test using SPSS version 20 and expressed as mean ± standard error of the mean (mean ± SEM). Values of $p < 0.05$ were considered statistically significant.

RESULTS

The result of administration of sub-convulsive dosage of PTZ on alternate days (Figure 1) showed the attainment of kindling around day 33 in the positive control group (PTZ). The neuroprotective effect of EA was seen here to decrease the seizure intensity in all the treated groups. In the PTZ + EA (15 mg/kg) and PTZ + EA (30 mg/kg) groups, seizure intensity was decreased to 3 on the Racine scale as compared to around 5 in the PTZ only group while in the PTZ + EA (60 mg/kg) and PTZ + PB (30 mg/kg) groups, seizure intensity decreased to 2.5 on

the Racine scaled as compared to 5 in the PTZ only ground around the period kindling was attained.

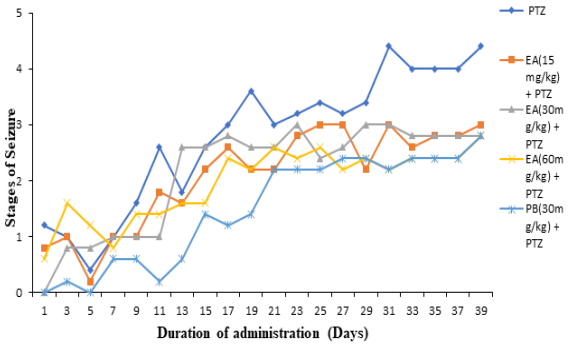

Figure 1: The flow chart for kindling activity in adult male rats administered with PTZ (35 mg/kg) and varying doses of EA (15, 30 & 60) mg/kg every alternate days for 40 days. PTZ = Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid, n = 60

The learning outcome of rats pre-administered with dosages (15, 30, 60) mg/kg of EA and administered with sub-convulsive dose (35 mg/kg) of PTZ (Figure 2) showed significant increase in learning ability in EA (60 mg/kg) + PTZ group (17.60 ± 3.31) when compared with the PTZ only group (43.00 ± 3.39), negative control (48.20 ± 4.03) and the standard drug (PB) + PTZ (60.00 ± 1.20) groups. However, the learning ability of the rats decreased significantly ($p < 0.05$) in EA (30 mg/kg) + PTZ group (49.60 ± 2.76) and PB (30 mg/kg) + PTZ group (60.00 ± 1.20) when compared with the PTZ group (43.00 ± 3.39).

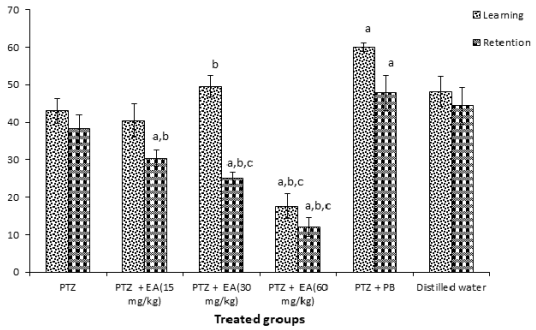

Figure 2: Effect of ellagic acid on long term spatial learning and memory in pentylenetetrazole – kindled rats using the elevated plus maze test. a = significant ($p < 0.05$) when compared to the PTZ group; b = significant ($p < 0.05$) when compared to the standard drug, (PTZ + PB) group; c = significant ($p < 0.05$) when compared to the negative control (distilled water) group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid.

In the retention (memory) test, rats given EA (30 mg/kg) + PTZ and EA (60 mg/kg) + PTZ showed significant ($p < 0.05$) increase in retention ability (25.00 ± 1.58) and (12.00 ± 2.55) respectively when compared with the PTZ only group (38.20 ± 3.74), negative control (distilled water) group (44.40 ± 4.86) and standard drug (PB) + PTZ group (47.80 ± 4.60). Meanwhile, the rats given 15 mg/kg EA + PTZ showed significant ($p < 0.05$) increase in retention too (30.40 ± 2.15) when compared with the PTZ only group (38.20 ± 3.74) and the standard drug (PB) group (47.80 ± 4.60). But there was significant decreased retention in the standard drug (PB) + PTZ group (47.80 ± 4.60) when compared with the PTZ group (38.20 ± 3.74) (Figure 2).

The result of the effect of EA on short-term working memory in rats (Figure 3) showed significant ($p < 0.05$) mean score of the percentage alternation in PTZ + EA (30 mg/kg) group (95.00 ± 4.00) and PTZ + EA (60 mg/kg) group (80.33 ± 8.37) when compared to the PTZ only group (56.76 ± 6.15). The mean scores of the other treated groups and the negative control (distilled water) showed increase in mean scores when compared to the control but are not significant.

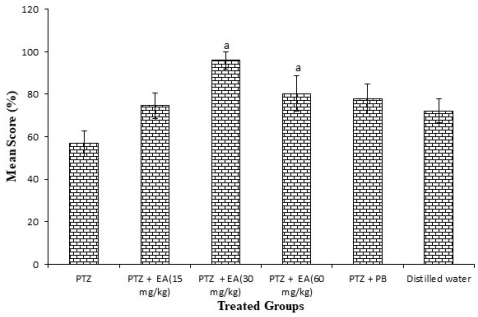

Figure 3: Effect of ellagic acid on short term working memory (mean percentage alternation score) in pentylenetetrazole – kindled rats using the y-maze test; a = significant ($p < 0.05$) when compared to the PTZ group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid.

The concentration of glutamate in PTZ – kindled rats (Figure 4) showed significant decrease in all treated groups. Glutamate concentration significantly decreased in PTZ + EA (15 mg/kg) group (5.58 ± 0.34), PTZ + EA (30 mg/kg) group (5.37 ± 0.23), PTZ + EA (60 mg/kg) group (5.72 ± 0.38), PTZ + PB group (3.59 ± 0.21) and negative control group (4.13 ± 0.42) when compare with the PTZ only group (8.50 ± 0.28). Also, when compared

with the standard drug (PTZ + PB) group (3.59 ± 0.21) there was significant increased glutamate concentration in the PTZ + EA (15 mg/kg) group (5.58 ± 0.34), the PTZ + EA (30 mg/kg) group (5.37 ± 0.23) and the PTZ + EA (60 mg/kg) group (5.72 ± 0.38). And when the comparison is made with the negative control (distilled water) group (4.13 ± 0.42), there was significant increase in the PTZ + EA (15 mg/kg) group (5.58 ± 0.34) and the PTZ + EA (60 mg/kg) group (5.72 ± 0.38).

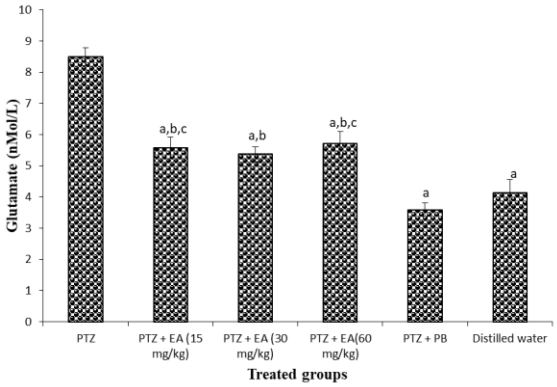

***Figure 4**: Effect of* Ellagic acid on the glutamate concentration in pentylenetetrazole – kindled rats; a = significant ($p < 0.05$) when compared to the PTZ group; b = significant ($p < 0.05$) when compared to the standard drug, (PTZ + PB) group; c = significant ($p < 0.05$) when compared to the negative control (distilled water) group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid

The result of the determination of GABA in PTZ – kindled rats (Figure 5) showed that the concentration of GABA in rat which were given 15 mg/kg of EA prior to PTZ administration (130.27 ± 3.20) nMol/L decreased significantly ($p < 0.05$) when compared to the concentrations in rats which received the standard drug (PB) (150.62 ± 1.52) but increased when compared to the distilled water group (104.92 ± 3.26) nMol/L. The concentration of GABA increased significantly ($p < 0.05$) in rats which received 60 mg/kg of EA prior to PTZ administration (218.51 ± 6.93) nMol/L when compared to the PTZ only group (135.16 ± 1.23) nMol/L, the standard drug (PB) group (150.62 ± 1.52) nMol/L and the distilled water group (104.92 ± 3.26) nMol/L. However, significant increased was observed in the concentration of GABA in rats which received the standard drug (PB) (150.62 ± 1.52) nMol/L when compared with the PTZ only group (135.16 ± 1.23) nMol/L but decreased significantly when the comparison is made between the distilled water group (104.92 ± 3.26) nMol/L and the PTZ only group (135.16 ± 1.23) nMol/L and the standard drug group (150.62 ± 1.52) nMol/L.

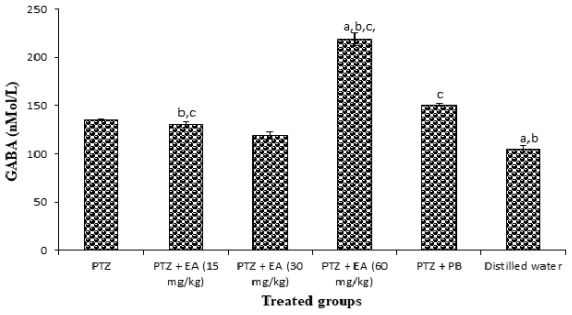

Figure 5: Effect of ellagic acid on gamma aminobutyric acid concentration in pentylenetetrazole – kindled rats; a = significant ($p < 0.05$) when compared to the PTZ group; b = significant ($p < 0.05$) when compared to the standard drug, (PTZ + PB) group; c = significant ($p < 0.05$) when compared to the negative control (distilled water) group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid

The concentration of MDA significantly decreased in all the treated groups when compared to the PTZ only group (figure 6).

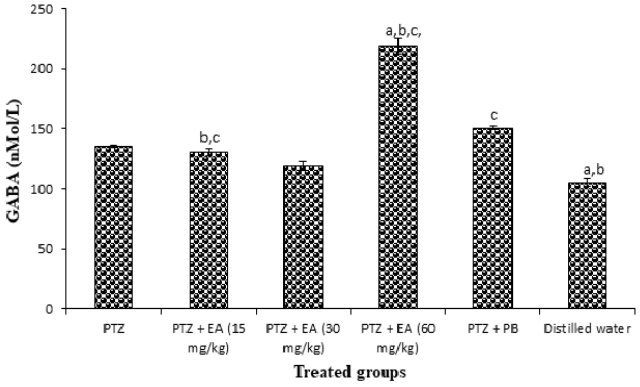

Figure 6: Effect of ellagic acid on malondialdehyde concentration in pentylenetetrazole – kindled rats; a = significant ($p < 0.05$) reduction when compared to the PTZ group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid

The concentration of SOD in the PTZ – kindled rats (Figure 7) showed significant increased in all the treated groups and the negative control (distilled water) group when compared with the PTZ only group. The highest increase was observed in the group that received 30 mg/kg of EA (2.20 ± 0.17) relative to the other groups.

This means that SOD concentration decreased drastically in the PTZ only group.

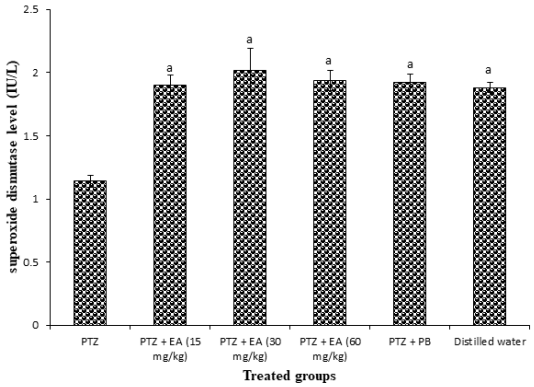

Figure 7: Effect of ellagic acid on superoxide dismutase concentration in pentylenetetrazole – kindled rats; a = significant ($p < 0.05$) when compared to the PTZ group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid

The result of CAT activity was determined in PTZ – kindled rats and the concentration of CAT (Figure 8) was significantly increased in rats that received PTZ with EA (30 mg/kg) (45.40 ± 0.68) IU/L when compared with the PTZ only group (41.40 ± 0.51) IU/L and the negative control (distilled water) group (40.60 ± 0.51) IU/L. Furthermore, CAT concentration increased significantly in the PTZ + EA (60 mg/kg) (48.80 ± 0.66) IU/L when compared with the PTZ only group (41.40 ± 0.51) IU/L, standard drug (PB) group (43.30 ± 1.03) IU/L and the negative control (distilled water) group (40.60 ± 0.51) IU/L.

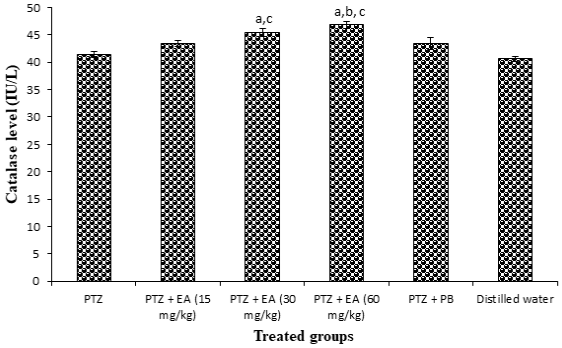

Figure 8: Effect of ellagic acid of on catalase concentration in pentylenetetrazole – kindled rats; a = significant ($p < 0.05$) when compared to the PTZ group; b = significant ($p < 0.05$) when compared to the standard drug, (PTZ + PB) group; c = significant ($p < 0.05$) when compared to the negative control (distilled water) group; PTZ= Pentylenetetrazole, PB = Phenobarbital, EA = Ellagic acid

DISCUSSION

It was observed in this study that sub-convulsive dose of PTZ administered on alternate days resulted into full kindling (stage 4 and 5 two or more consecutively) in the PTZ group only. This showed that PTZ- treated rats had significant increase in seizure score. This is in agreement with the finding of Agarwal et al. (2011), who studied the effect of PTZ-kindling in mice and found out that PTZ-treated mice showed increase in seizure score. The kindling activity of PTZ as observed in the PTZ group showed that PTZ is a strong convulsant and might have activated glutamate release. Glutamine release cause a blockage of $GABA_A$ receptors thereby leading to recurrent seizures. Dhingra and Jangra (2014) studied the acute and chronic effects of EA in mice following PTZ convulsions and found that PTZ triggers convulsions by exerting an inhibitory effect on GABA-mediated chloride ion influx via an allosteric interaction at chloride ion channels, which leads to the activation of excitatory neurons, excitotoxicity and ultimately seizures. They demonstrated that acute and chronic administration of EA delayed the onset of convulsions, while also reduced the duration of tonic and clonic convulsions and mortality in PTZ conditions.

In the result for learning and memory using the elevated plus maze, it was found that there was a significant decrease in learning latency of the EA (60 mg/kg) group when compared to the other learning latencies. There were also decreased retention latencies in all the EA - treated groups; a clear indication that EA enhanced the learning and memory ability of the rats. Though the seizure in this study did not cause significant impairment in memory, yet EA improved on the learning and retention of the rats. EA – treated groups showed significant reduction in transfer latency for both learning and retention. This proved EA to be effective not only in mitigating seizure and oxidative stress but also in enhancing learning and memory. EA, a flavonoid, has been found to have neurogenic potentials. This finding agrees with the work of Kiasalari *et al*. (2017) who stated that EA pre-administration could dose-dependently improve learning and memory via neuronal protection and at molecular level through mitigation of oxidative stress and acetylcholinestrase (AChE) activity. The latencies in the PB and distilled water groups were higher than those in the PTZ-administered group. The rats in the PTZ group were kindled by PTZ and this kindling excited the animals leading to higher activity and faster movement in the animals which eventually

reduced their latencies when compared with the PB and distilled water groups. This finding contradicts that of Hannesson *et al.* (2001) who explained that kindled and control rats exhibited comparable levels of activities as shown by similar distances travelled.

Percentage alternation mean score in the Y-maze showed a significant increase in mean score observed in the PTZ + EA (30 mg/kg) group when compared to the PTZ group. This implies that EA enhanced the working memory in rats at 30 mg/kg as compared to the other dosages and the standard drug PB (30 mg/kg). This is consistent with the finding of Macready et al. (2009) that flavonoids, like EA, improves spatial working memory in Y-maze task and that supplementations in rats enhanced spatial working memory and higher levels of brain -derived neurotrophic factor (BDNF) in the hippocampus. But Hannesson *et al*. (2001) disagreed by stating that dorsal hippocampus kindling altered acquisition performance on a Y-maze discrimination task. This memory enhancing ability of EA could be due to its strong antioxidant capacity as PTZ is known to cause oxidative stress in rodents which can lead to brain damage induced by oxidative process and plays a crucial role in the pathogenic consequences of seizures. This improvement in learning and memory could also be as Chao *et al.* (2009) noted that EA possesses non-enzymatic antioxidant activity such as scavenging free radicals, and enzymatic antioxidant activity such as increasing protein level of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione and glutathione peroxidase (GSH-GP_X). Also, according to Bansal and Kumar (2018), EA modulates the brain mono-aminergic and GABAergic transmission that have profound effects on learning and memory. It reduces Aβ40-42-plaque deposition in brain and improved in memory of rodents. EA inhibits the AChE activity and thereby up-regulates the cognitive abilities through acetylcholine. The memory improvement could be attributed to AChE-reduction activity of EA as reported by Ferreres *et al.* (2013) that brain tissue showed a marked decrease in brain AChE activity in the presence of EA thereby increasing the concentration and duration of action of acetylcholine which play crucial role in memory.

The findings of Farbood *et al*. (2015) and Mansouri *et al*. (2016) are in agreement with our findings that the administration of EA dose-dependently improved learning and spatial memory deficits and also that there were improvements in memory in various models of cognitive deficits. EA is capable to pass through the blood–brain barrier at small quantity. In this respect, EA has been detected in the rat brain after its administration and it could be considered a factor in the neuroprotection associated with the intake of certain fruits and foods (Kiasalari *et al*., 2017).

This present study has shown that PTZ kindling led to significant increase in MDA concentration in the PTZ group only when its concentration in all the treated groups decreased significantly, showing that oxidative stress might be involved in the pathogenesis of kindling. And this indicated that kindling produced oxidative stress in the rats. This is supported by the works of Liu *et al*. (2012) which showed that there was increase in MDA concentration as a result of PTZ kindling. Frantseva *et al.* (2000) also have shown that seizure leads to the production of oxygen radicals, increasing lipid peroxidation in both hemispheres and probably these radicals are involved in the seizure and convulsion-induced neuronal death.

Ellagic acid based on this research is a potent agent with antiepileptogenic potential because none of the EA treated groups was kindled throughout the period of administration. EA could be responsible for this development since MDA concentration decreased significantly in all the EA groups, showing its anti-seizure potentials. This finding agrees with that of Wang *et al*. (2008) who stated that EA has the affinity to bind with benzodiazepine sites on the $GABA_A$-receptor where PTZ acts to elicit its convulsant effect. The benzodiazepine site is one of the many binding sites on the $GABA_A$ receptor.

Antioxidant enzymes activities increased in the E.A administered groups; while SOD activity increased in all the treated groups, CAT increase increased in the rats that received 30 mg/kg EA, 60 mg/kg EA while GPx activity increased only in the EA 60 mg/kg group. These are indications that EA acted by enhancing the activities of the antioxidant enzymes to mitigate the effect of the oxidative stress. This is in agreement with many findings including that of Galano *et al*. (2014), which showed that EA provides protection against oxidative stress and lipid peroxidation. This feature distinguishes EA from most antioxidants and makes it to provide continuous protection against oxidative stress through a free radical scavenging cascade. It is also worthy to note that the

concentration of all the antioxidant enzymes decreased significantly in the PTZ administered rats when compared with the EA treated groups as already revealed by Silva *et al*. (2009) that convulsions followed by an increase in lipid peroxidation in the brain tissue decrease the levels of antioxidant enzymes. This finding is also agreed with the work of Khalili *et al*. (2011) which showed that there was significant increase of MDA as an index of lipid peroxidation and significant reduction of antioxidant enzyme SOD in the PTZ-induced kindled group leading to excess production of free radicals and existence of oxidative stress in the brain. Similarly, Kumar *et al*. (2016) showed that levels of SOD, CAT and GSH were significantly decreased in the PTZ-treated mice and this agreed with our findings. Chen *et al*. (2018) have also shown that the activities of antioxidant enzymes were effectively increased by EA supplementation, which also significantly reduced the production of MDA in the brain. EA is a potent antioxidant and counters the effects of oxidative stress at late stages of pathogenicity by aiding the regeneration of cellular antioxidants such as glutathione peroxidise (GSH) and ascorbate and by the activation or the induction of genes responsible for expressing enzymes such as SOD, CAT, glutathione S-transferase (GST), Nicotinamide adenine dinucleotide phosphate (NADPH) - quinone oxidoreductase and others which are involved in managing the oxidative stress. And hence provides better protection against oxidative stress and lipid peroxidation than Vitamin E and succinate.

The result for assessment of glutamate level showed that glutamate concentration decreased in all the EA - treated groups compared to the PTZ administered group and reduced significantly in the phenobarbital group as compared to the EA groups. Glutamate is the principal excitatory neurotransmitter in the brain and acts on ionotropic and G-protein-coupled metabotrophic glutamate (mGlu) receptors. According to Moldrich *et al*. (2003), excessive glutamatergic neurotransmission is understood to be one of the primary metabolic and pathological mechanisms behind the aetiology of numerous types of epilepsy. Therefore, reduction in the concentration of glutamate in the EA treated groups showed that EA could have blocked the release or action of glutamate, meaning it might have acted by blocking its release from nerve terminal or blocked the glutamate receptors. It has been stated that glutamate is the main excitatory neurotransmitter in the three-circuit loop of the hippocampus and also the whole brain. Any increase or decrease of glutamate content will alter excitability of the related circuits (Stanley and Fadel, 2011).

Gamma amino butyric acid concentration increased significantly in only the 60 mg/kg EA - treated group when compared to the PTZ control group and the EA - treated groups. The primary inhibitory neurotransmitter is GABA and it exists widely in central nervous system of mammals. The GABA receptors mediate inhibitory neurotransmission to prevent neurons from being overexcited in adult brain (Kong *et al*., 2014). The increase seen in GABA concentration of the EA group is an indication that as glutamate concentration reduced, GABA concentration increased, thereby creating an imbalance. This agreed with the work of Diniz *et al*. (2015) that an imbalance exists between excitatory and inhibitory neurotransmitters in the pathophysiology of epilepsy. In the same vein, Dhingra and Jangra (2014) showed that both acute and chronic administration of EA significantly reversed PTZ-induced convulsions and decrease in brain GABA levels. PTZ is known to block the chloride ionopore coupled to GABA receptors, thereby eliciting seizures and free radical generation. Therefore, the increased GABA in EA - treated groups especially the 60 mg/kg group indicated that EA could have blocked the receptors, making it hard for the PTZ to bind, thereby, increasing the concentration of GABA.

CONCLUSION

This study showed that EA pre - administered in PTZ – kindled rats has a neuroprotective effects through reduction in seizure intensity by decreasing glutamate and increasing GABA concentrations. There was also an increase in antioxidants activities which could have mopped up ROS generated in the rats during kindling leading to enhanced learning and memory.

LIMITATION OF THE STUDY

Due to paucity of funds and lack of some electrophysiology equipment such as electroencephalograph, we couldn't conduct electroencephalography as well as channels and receptors studies.

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