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Assessing the Anti-Epileptic and Anti-Nociceptive Potentials of *Pepperomia pellucida* in Pentylenetetrazol-Induced Epileptic Seizure in Mice

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Abstract

Background: Epilepsy has become one of the major public health problems in the world is characterized by seizures resulting from abnormal electrical discharges in the brain. Hence, this study to assess the anti-epileptic and antinoceptive potentials of *Pepperomia pellucida* in pentylenetetrazol-induced epileptic mice model. **Methods:** Forty (40) Swiss male albino mice weighing 21 to 35kg were randomly assigned into four groups (A to D) ten mice each and treated as follows: Group A served as the control, Group B received pentylenetetrazol 35mg/kg intraperitoneally, Group C received pentylenetetrazol 35mg/kg intraperitoneally + *peperomia pellucida* 2mg/kg orally, and Group D received pentylenetetrazol 35mg/kg intraperitoneally + Diazepam 0.001ml/kg orally. Pentylenetetrazol 35mg/kg intraperitoneally was used to induce epilepsy and the animals were considered epileptic after few minutes with a jerky movement of the tail and limb progressing to the entire body. All animals had access to food and water *ad libitum*. After administration for 14 days, epilepsy was induced and then neurobehavioral studies for nociception was assessed using the formalin test. **Results:** pentylenetetrazol 35mg/kg had significant epileptic seizures scores as evidenced by increased

duration of seizures, frequency of jerks, onset of tonic seizures and onset of seizures and also had significant edema of the animals' paw. *Peperomia pellucida* significantly ameliorated the epileptic seizure and reduced the edema of the animals' paw induced by the pentylenetetrazol. IBM SPSS statistical software version 20, was used to analyze the data $p<0.001$. **Conclusions:** It is based on these results, we draw the conclusion that *Peperomia pellucida* may possess anti-epileptic and anti-nociceptive properties that resulted in the significant reduction in the epileptic seizures and edema of the animals' paw in the *Peperomia pellucida* treated group.

Keywords: Epilepsy, *Peperomia pellucida*, Seizure, Antinociception

INTRODUCTION

Seizures caused by aberrant electrical discharges in the brain are the hallmark of epilepsy, which has emerged as one of the world's most significant public health issues (Thijs *et al,* 2019).

Numerous factors impact the quality of life for individuals with epilepsy (Kim *et al*., 2020; Taylor *et al*., 2011).

Typically, seizure attacks are unpredictable. People with epilepsy often feel stigmatized because of their rapid loss of consciousness, limb convulsions, changed psychological state, and occasional incontinence (Kaddumukasa *et al*, 2016; Chakraborty *et al*, 2021).

The frequency of seizures, length of illness, quantity of antiseizure medications, and adverse drug reactions are some of the characteristics that have been shown to impact the quality of life for individuals with epilepsy (Mosaku *et al*, 2006; Zhao *et al*, 2011; Blond *et al*, 2016).

Antiepileptic medications (AEDs) are the standard treatment for epilepsy; its goals are to reduce seizures and enhance patients' quality of life (Perucca & Gilliam, 2012). AEDs are linked to serious adverse effects such hepatotoxicity, skin issues, and cognitive impairment, and their effectiveness varies from person to person (Löscher & Schmidt, 2011).

Diazepam is one of the most widely used AEDs for treating epilepsy. It is quite successful at controlling epileptic seizures, but it has drawbacks such sedation, tolerance, and reliance (Treiman, 2001).

Concern over these areas has really grown recently, and *Peperomia pellucida* is one plant that may aid with herbal remedies that target these problems.

In many regions of Asia and South America, the little herbaceous plant *Peperomia pellucida*, sometimes referred to as shiny bush or silver bush, has long been utilized in traditional medicine.

As an analgesic, anti-inflammatory, and anti-convulsant, it has been widely utilized in traditional medicine (Moura *et al.,* 2016).

According to Egwuche *et al.* (2011), the plant's pharmacological effects are thought to be caused by a number of active chemicals, including tannins, alkaloids, cardenolides, and saponins.

In animal models of pain and epilepsy, *Peperomia pellucida* has demonstrated encouraging outcomes (Moura *et al,* 2016). Wei *et al.* (2011) looked at the plant's antibacterial properties in their study.

For many years, researchers have utilized pentylenetetrazol, also known as pentylenetetrazole, a non-specific GABAergic agonist and convulsant medication, to investigate epilepsy and other brain problems (Löscher, 2002).

Pentylenetetrazol-induced seizures were once used to diagnose epilepsy, but their usage as a diagnostic method decreased when electroencephalography was developed (Kaminska *et al*, 2013).

The dangers of stopping the drug are highlighted by the fact that pentylenetetrazol withdrawal can cause significant withdrawal symptoms, such as anxiety and seizures (Grootendorst & Kasteleijn, 2013).S

METHODOLOGY

Procurement of Test Substances

1. **Chemicals:** The Pentylenetetrazol was purchased from Sigma- Aldrich Limited, Canada while Diazepam was purchased from Bez Pharmacy, Calabar, Cross River State. All reagents and chemicals used for this study were of analytical grade.
2. **Extraction of the ethanolic extract of *Peperomia pellucida***

Peperomia pellucida leaves were obtained from the botanical garden, University of Calabar, Calabar. A botanist authenticated a sample of the plant (Voucher number - Bot/Herb/UCC/0984). The leaves were washed under running tap water and made free

from dirt and sand. The fresh air-dried leaves was powdered in an electric kitchen blender and 300g of the powdered plant material was obtained. Preparation of the ethanolic extract was by the method of Klyushnichenko *et al.,* (1996). 300g of the powered leave extract of *Peperomia pellucida* was completely immersed in 30% of ethanol and shaken vigorously. It was allowed to stand for (24) twenty four hours at room temperature and stirred at intervals. After 24 hours, the extract was filtered severally especially using NO1 What Mann filter paper of pore size 0.45 micrometer and funnel, the filtrate was then allowed to concentrate using rotatory evaporator and Astell Hearson oven was further used to dry the concentrate at 40 – 45^0c. This was to ensure complete evaporation of the extract to a pasty black residue.The leave extract of *peperomia pellucida* was collected with the aid of a spatula into a container and was measured using an electric weighing balance. 35g of *peperomia pellucida* paste was obtained at the end of preparation and was kept in a cool dry place at room temperature.

3. Laboratory Animals: Forty (40) swiss male mice of 10 weeks weighing 21 to 35kg were used for this study. The animals were housed in the Department of Physiology Animal House, University of Calabar, Nigeria. Standard animal cages (435 x 290 x150mm) with wood shavings as bedding were used in housing the animals (5 mice per cage). They were given *ad libitum* access to feed (Flourmill Calabar, Cross River State, Nigeria) and fresh water, and exposed to 12/12-hr light/dark phase. The animals were acclimatized for a period of one week and kept in line with laid - down ethics for animal care approved by the National Committee for Research Ethics in Science and Technology (NENT), 2018. Before the commencement of this research, ethical approval was obtained from the University of Calabar animal ethics committee, which aligned with the standard guidelines for the use of laboratory animals outlined by the World Health Organization. The study was permitted with ethical clearance with approval number (Approval No.FARE C/PA/ [UC/050]/181PHY318)

4. Experimental Design

The animals were randomly allotted into 4 different groups (n=10). At the expiration of the one week of acclimatization, *peperomia pellucida* 2mg/kg and Diazepam 0.001ml/kg was administered orally to treatment groups C and D for 14 days. Thereafter, Pentylenetetrazol 35mg/kg intraperitoneally was administered to Groups B and C. The animals were

considered epileptic after administration of pentylenetetrazole few minutes after with a jerky movement of the tail and limb progressing to the entire body.

(Dose per mice outlined in Table 1), once daily, to animals in treatment groups B and C using the doses outlined in Table 1. Whereas the control group was given feed and 0.5ml normal saline as a vehicle throughout the experimental duration.

Thereafter, the animals were subjected to epileptic seizure scoring and behavioral testing to assess behavioral changes at the expiration of the treatment.

Table 1. Study Design and Drug and Extract administration

GROUPS	NO. OF MICE	TREATMENT
GROUP A	10	Feed + 0.5ml of normal saline as a vehicle throughout the experimental period
GROUP B	10	35mg/kg bw of Pentylenetetrazole
GROUP C	10	35mg/kg bw of Pentylenetetrazole + 2mg/kg bw of *peperomia pellucida*
GROUP D	10	35mg/kg bw of Pentylenetetrazole + 0.001ml/kg bw of Diazepam

5. Induction of epilepsy

Epilepsy was induced by intraperitoneal injection of 35mg/kg of pentylenetetrazole, freshly prepared in normal saline before laboratory testing.

The animals were considered epileptic after administration of pentylenetetrazole few minutes after with a jerky movement of the tail and limb progressing to the entire body. The parameters scored included:

a. Onset of seizures: This is when the seizures starts in the brain
b. Frequency of jerks: This is the brief shock-like jerks of a muscle or a group of muscles that occur during seizures in epilepsy
c. Duration of seizures: is how long the seizure lasted
d. Onset of tonic seizures

6. Protocol test for accessing nociception (pain)

Formalin test was carried out as previously described by Sawada *et al,* 2014 with some modifications. The formalin solution is the algic agent. Licking is a rapid response to painful chemical stimuli that is a direct indicator of nociceptive threshold. The time that the animal spent licking the injected paw and the swelling of the paw which is considered indicative of pain, was recorded for 30 minutes immediately following formalin injection. A significant reduction in the licking time and reduction in the swelling of the paw was considered indicative of antinociceptive activity

Test Procedure

a. Formalin (usually 1-5%) is injected subcutaneously into the hind paw of a mice

b. The animal's pain response is observed and recorded.

c. The anterioposterior and mediolateral paw diameter before the test procedure is recorded

Parameters Measured: Edema (swelling) and inflammation (the diameter of the paw after 12 hrs)

7. Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean (M ± S.E.M). The result was analyzed using one-way Analysis of variance (ANOVA) followed by post-hoc multiple comparison test to compare level of significance between other groups and the control. SPSS version 20 and Microsoft Excel were used for the analysis. The level of significance was set at $P<0.001$.

RESULTS

1. **Comparison of** Onset of seizures

   The onset of seizure in the PTZ + *Peperomia pellucida* treated group took a longer time to begin when compared to the pentylenetetrazol (PTZ) treated group and this was significantly different at ($p<0.001$). This is shown in fig. 1

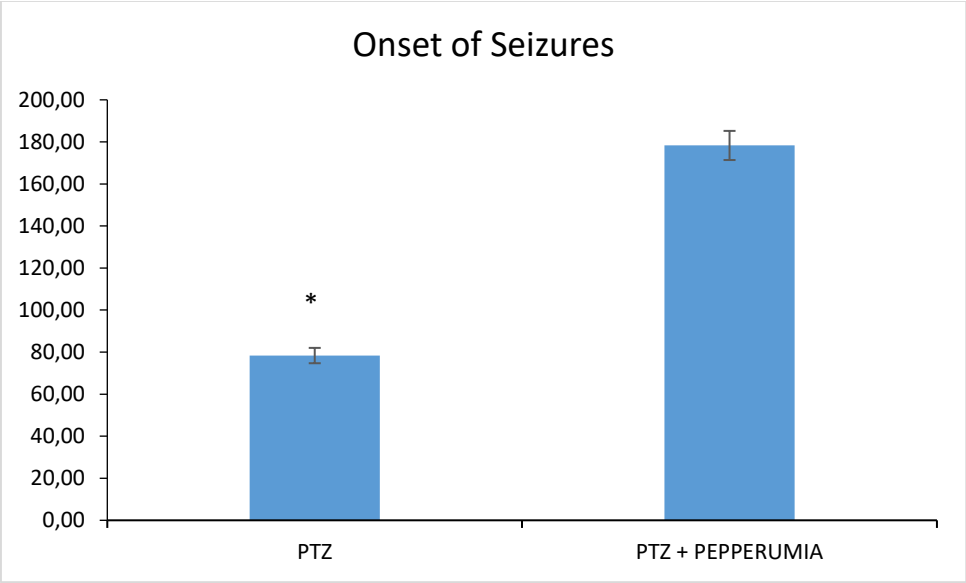

Figure. 1: Onset of Seizures

n=10; mean ± SEM Independent sample *t*-test, *=p<0.001 significance difference between the two groups. **PTZ**= Pentylenetetrazol

2. **Comparison of frequency of jerks**

The frequency of jerks in the *Peperomia pellucida* treated group was significantly decreased (p<0.01) when compared to the PTZ treated group which had an increased frequency of jerks. This is shown in fig. 2.

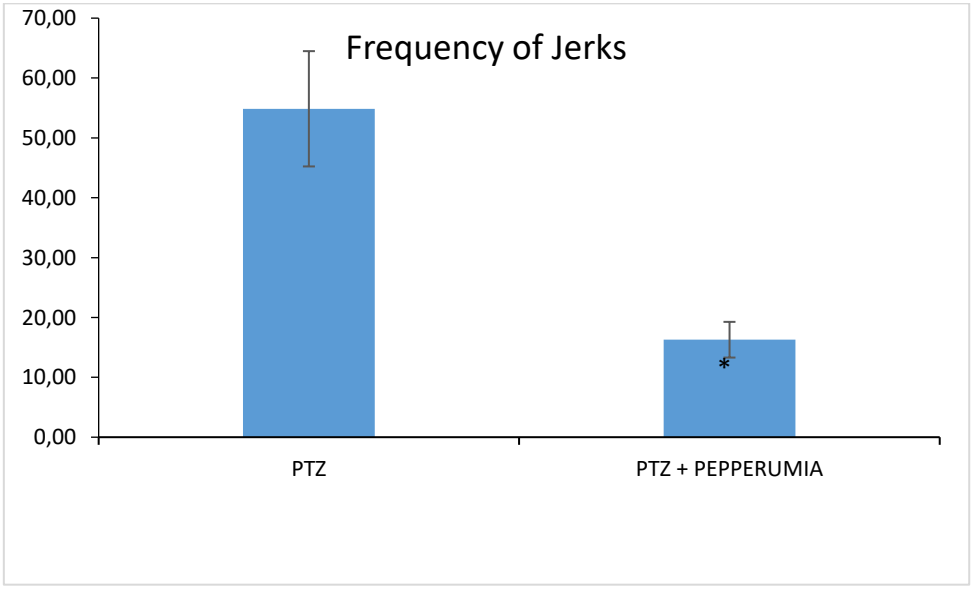

Figure 2: Frequency of jerks

n=10; mean ± SEM Independent sample *t*-test, *=p<0.01 significance difference between the two groups. **PTZ**= Pentylenetetrazol

3. **Comparison of duration of seizures**

The result showed a significant decrease (p<0.001) in the seizure duration in the *Peperomia pellucida* treated group when compared to the PTZ treated group. This is shown in Fig. 3

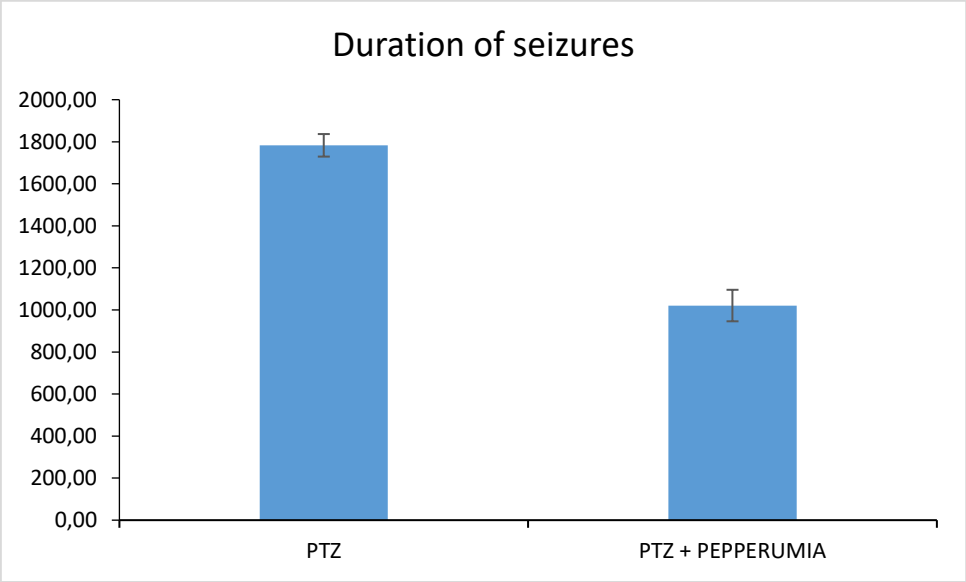

Figure 3: Duration of seizures

n=10; mean ± SEM Independent sample *t*-test, *=p<0.001 significance difference between the two groups. **PTZ**= Pentylenetetrazol

4. **Comparison of onset of tonic seizure**

In the onset of tonic seizure, there was a significant decrease in the *Peperomia pellucida* treated group when compared to the PTZ treated group. This is shown in Fig. 4

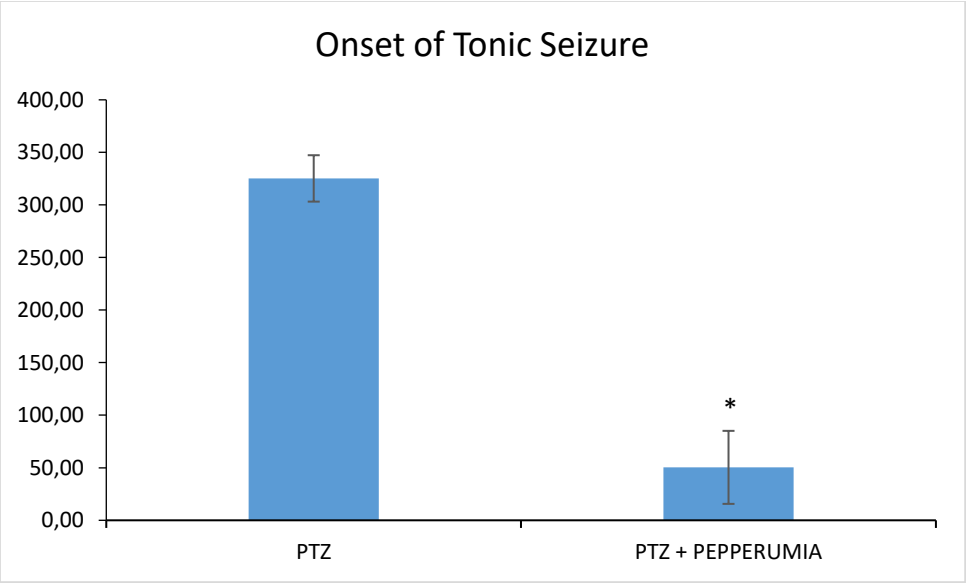

Figure 4: Onset of tonic seizure

n=7; mean ± SEM Independent sample *t*-test, *=p<0.001 significance difference between the two groups. **PTZ**= Pentylenetetrazol

5. **The result of anteroposterior paw diameter before and after**

Results of the before and after anteroposterior paw diameter measurements revealed that the PTZ + *Peperomia pellucida* treated group had the least anteroposterior paw diameter (P>0.001) when compared to the control, PTZ, and PTZ + Diazepam treated groups. This is shown in Figure 5

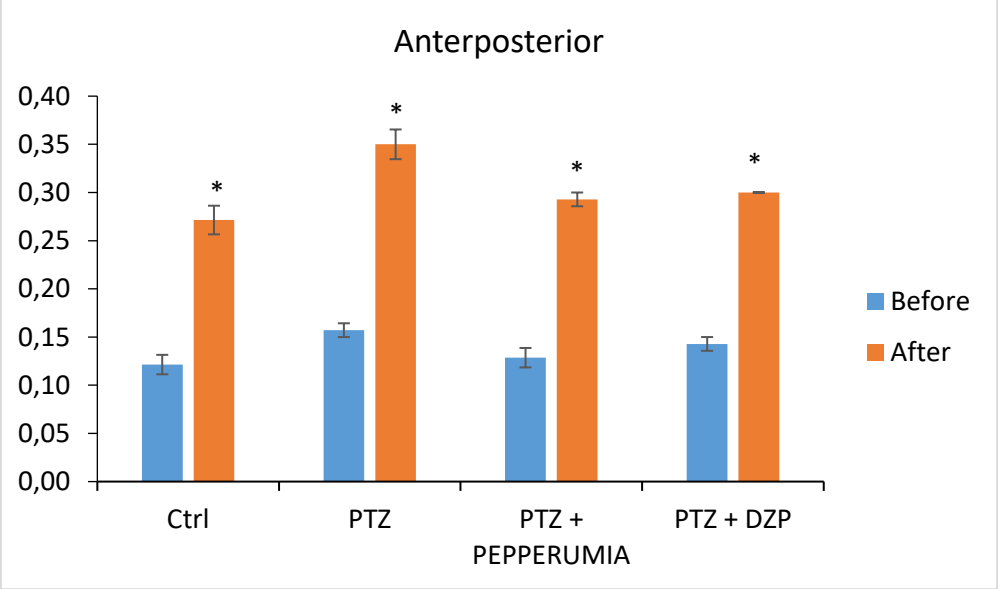

Figure 5: Anteroposterior paw diameter before and after

n=7; mean± SEM; paired *t*-test, *=p<0.001 significance difference when anteroposterior diameter before and after test was compared. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

6. **The result of mediolateral paw diameter before and after**

Results of the before and after mediolateral paw diameter measurements revealed that the PTZ + *Peperomia pellucida* treated group had the least mediolateral paw diameter (P>0.001) when compared to the control, PTZ, and PTZ + Diazepam treated groups. This is shown in Figure 6.

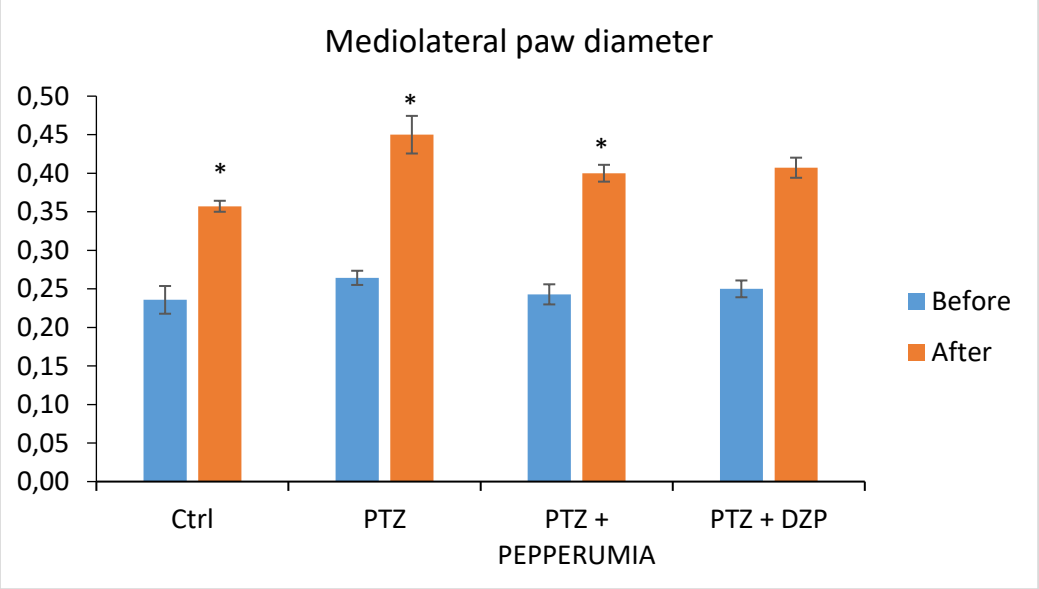

Figure 6: Mediolateral paw diameter before and after

n=7; mean± SEM; paired *t*-test, *=p<0.001 significance difference when mediolateral diameter before and after test was compared. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

DISCUSSION

The research work was aimed at assessing the anti-epileptic and anti-nociceptive potentials of *Pepperomia pellucida* in pentylenetetrazol-induced epileptic seizure in mice.

Epilepsy was inducted and formalin test was used to assess nociception (pain) in the animals.

In this research, it is observed that in the epileptic seizure scoring, the Onset of seizure which is when the seizure starts in the brain in the pentylenetrazol (PTZ) treated group took a shorter time to begin. While the *peperomia pellucida* treated group had a longer time for the seizure to begin which was significantly different when compared to the PTZ group.

Frequency of jerks is brief shock-like jerks of a muscle or a group of muscles that occur during seizures in epilepsy. The Frequency of jerks in the PTZ treated group was significantly increased at P<0.001 while in the *peperomia pellucida* treated group the Frequency of jerks was significantly decreased.

Duration of seizures is how long the seizure lasted. In the PTZ treated group there was significant increase in seizure duration, but reduced duration in seizures was observed in the *peperomia pellucida* treated group at P<0.001.

Similar trend was also observed in the onset of tonic seizure were the PTZ treated group had a significant increase in onset of tonic seizure, while the *peperomia pellucida* treated group had a great reduction in the onset of tonic seizure at P<0.001.

Though, there's no evidence to ascertain what mechanism could be responsible for the reduction of the epileptic seizure scoring evidenced in the *peperomia pellucida* treated group but this action could be attributed to the presence of the phytochemical constituents such as tannins, saponins, alkaloids etc Egwuche *et al,* 2011

In the formalin test, the *peperomia pellucida* treated group had a significant p<0.001 reduction in before and after mediolateral and anteroposterior paw diameter measurements indicating that the PTZ + *Peperomia pellucida* had a significant low edema development. This antinociceptive effect of *Peperomia pellucida* is consistent with the finding of Amanda Pàmela *et al* 2020, where, it was elucidated that pellucidin A, a component of *Peperomia pellucida* most likely promoted the antinociceptive activity by peripheral mechanisms and interactions with COX and nitric oxide pathways and also to further buttress the reduction in paw edema, the work of De Fatima *et al*, 2004, indicated that the aqueous extract of *Peperomia pellucida* provided inhibition of edema at a dose of 400mg/kg approaching the activity of indomethacin 10mg/kg.

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